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Diversificación de las especies altoandinas del grupo *Pristimantis conspicillatus* (Anura: Brachycephalidae): fauna anura primitiva o derivada de la fauna de tierras bajas

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The present thesis has been written in plural instead of singular first person in order to facilitate further preparation of this document for publication that will be multi-authored. However, all of the work, failures and conclusions are deemed to be charged to me as author of the present thesis. I assume full responsibility about this thesis in its integral form.

Abstract

One of the fundamental goals of biology is to understand the patterns and processes underlying diversification. These can be studied using phylogenies. Through phylogenies, it is possible to reconstruct the evolutionary history of a group, even in the absence of paleontological information. Given the advances in molecular biology, it is now possible to generate phylogenies for a large number of groups. Since 2005, the amphibian phylogenies involving molecular data increased markedly in number, presenting dramatic change in the systematic and taxonomy of the most groups.

The genus *Pristimantis* Jiménez de la Espada, 1870, was resurrected for the molecular clade including most former South American *Eleutherodactylus* Duméril & Bibron, 1841 by Heinicke *et al.*, 2007 and Hedges *et al.*, 2008. The genus *Pristimantis* was subdivided in several species groups and series by Hedges *et al* (2008). The former *E. conspicillatus* group is now *Pristimantis conspicillatus* group, this group contains most of the species recognized in the "*E conspicillatus*" group by Lynch & Duellman (1997). Hedges *et al.* (2008) redefined the content of the *E. conspicillatus* group proposed by Lynch (1986, 1994) and Lynch & Duellman (1997), and allocated 37 species into the group and showed support for the monophyly of this species group. Padial *et al.* (2014), in their molecular systematics of terraranas, found that *P.*

conspicillatus group could be rendered monophyletic by making a few changes. Members of the *Pristimantis conspicillatus* group have a large and interesting distribution. These species occur from Costa Rica to Bolivia across the Andean cordillera, the Amazon and Cerrado, the Guianas and in the Atlantic Forest; and have a range from the lowlands up to about 3000 m.a.s.l. Given that species of the *P. conspicillatus* group are distributed in the highlands and lowlands, we used frogs of the *P. conspicillatus* group as a model system to address the following questions: 1) Are lowland species of *P. conspicillatus* group more closely related to other lowland species or to highland species?, 2) What was the most probable ancestral distribution area for *P. conspicillatus* group?, 3) Does the radiation of *P. conspicillatus* group follow a south to north pattern of speciation?, 4) Can the *P. conspicillatus* diversification be attributed to allopatric/vicariant scenario?.

To evaluate mode and area relationships of the diversification of *P. conspicillatus* species group, we reconstructed phylogenetic relationships using the maximum possible number of species that have been assigned to the *P. conspicillatus* group *sensu* Lynch 1994, Lynch & Duellman, 1997, Hedges *et al.* 2008 and Padial *et al.*, 2014. We used an extensive taxon (617 terminals), data set of 17.365 aligned sites of mitochondrial and nuclear DNA, and 32 morphological characters. Phylogenetic analyses were performed using parsimony analysis and maximum likelihood (ML).

Pristimantis conspicillatus group was recovered as a monophyletic under both parsimony and ML analysis. We provide a new definition of the *P. conspicillatus* group based on external morphology and cranial osteology. With a phylogenetic hypothesis for a monophyletic group containing a mixture of lowland and montane species, coupled with detailed geographic and elevational range data, it is possible to understand diversification of montane fauna.

The present study illustrate how the different geological and climatic events of the Neotropics shaped, at different levels of the phylogeny. According to the new circumscription of the *P. conspicillatus* group showed in this study, most of the species are distributed in the Amazon basin and Andean slopes of Peru and Bolivia and a few species are distributed on northern Andes. Our results identify Amazonia as the most probable ancestral range of the most recent common ancestor of *P. conspicillatus* group. Amazonian origin of the *P. conspicillatus* group was followed by several events of colonization to other habitats. The highland species of *P. conspicillatus* group are more related to lowland species, where the montane species are derived, supporting the hypothesis of montane regions largely as 'species pumps'. Within the *P. conspicillatus* group there is not a generalized patter of speciation, ecological speciation and vicariance models explain the diversification of the *P. conspicillatus* group.

Keywords: Eleutheretodactylus, conspicillatus group, phylogenetic analysis, Andes, Biogeography, taxonomy, morphology.

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Chapter 1. General Introduction

The genus *Pristimantis*

Before 2005, the genus *Eleutherodactylus* Duméril & Bibron, 1838 was the most species rich of all vertebrate genera (Hedges, 1989; Lynch & Duellman, 1997), with over 700 Neotropical species. Because of this remarkable diversity, the genus *Eleutherodactylus* was subdivided into various subgenera: *Craugasto*r, *Euhyas*, *Eleutherodactylus* (*sensu stricto*), *Pelorius* and *Syrrophus*, and into series and several species groups (Lynch, 1976; Lynch & Duellman, 1980; Savage 1987; Hedges, 1989; Lynch & Duellman, 1997; Lynch, 2001), most of these groups were phenetic instead of phylogenetic groups.

Frost *et al.* (2006) based on molecular data indicated that the genus *Eleutherodactylus* was traditionally understood, this genus does not belong family Leptodactylidae, now this genus belong to the family Brachycephalidae. Furthermore, Frost *et al*. (2006) divided *Eleutherodactylus* (*sensu lato)* into genera: *Craugastor*, *Eleutherodactylus*, *Euhyas* and *Syrrophus*, and showed that *Eleutherodactylus* (*sensu stricto*) might not be monophyletic. Frost *et al.* (2006) recognized as monophyletic *Craugastor*, *Syrrhophus*, as dubiously monophyletic "*Euhyas*" and "*Pelorius*". They also found *Eleutherodactylus* (*sensu lato*) to be rampantly nonmonophyletic and considered *Eleutherodactylus* (*sensu stricto*) as demonstrably nonmonophyletic, because the genera of Brachycephalidae as *Barycholos*, *Brachycephalus and Oreobates* fall within *Eleutherodactylus* (*sensu stricto*). Heinicke *et al.* (2007) split the genus *Eleutherodactylus* Duméril & Bibron, 1841, into three larger and other smaller genera, and resurrected the genus *Pristimantis* Jiménez de la Espada, 1870, containing *Eleutherodactylus unistrigatus* species group, *Eleutherodactylus conspicillatus* species group and 13 other species groups. Hedges *et al.* (2008) offered a new taxonomic revision of the former "eleutherodactylines", erecting a new taxon (Terrarrana) containing four families: Brachycephalidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae, the genus *Pristimantis* was assigned to the Strabomantidae. Hedges *et al.* (2008), recognized three subgenera within *Pristimantis*: *Hypodictyon*, *Yunganastes* and *Pristimantis*. The first one includes species previously placed mostly in the "*Eleutherodactylus cruentus*" and "*Eleutherodactylus cerasinus*" species groups, and the second one mostly includes species assigned to the "*Eleutherodactylus fraudator*" species group. The subgenus *Pristimantis* contains the species placed previously in the enormous "*Eleutherodactylus unistrigatus*" species group, as well as species placed in fifteen other species groups, which they were previously recognized by Lynch & Duellman (1997) and others have been proposed subsequently. In some cases, the phylogenetic trees showed by Hedges *et al.* (2008), were discordant between molecular and morphological definitions of groups and for this reason, it was not possible to allocate species lacking sequence data to groups defined only in the molecular phylogeny. Furthermore, many of some species groups were considered as not monophyletic, except for the *Pristimantis conspicillatus* and *P. peruvianus* species group, having strong support from the molecular phylogeny.

Pyron & Wiens (2011) in their study of genbank sequences confirmed the monophyly of *Pristimantis* and suggested a family-level revision of that taxonomy, they found Strabomantidae to be no monophyletic and the former Craugastoridae (now Craugastorinae) embedded within it; for this reason, they placed the elements of Strabomantidae within Craugastoridae. Pinto-Sánchez *et al.* (2012) added new sequences and provided a new terrarana molecular phylogeny that suggests that the subgeneric taxonomy of *Pristimantis* is clearly flawed and that almost none of the species group so far suggested are monophyletic.

Padial *et al.* (2014), provided a new taxonomy of Terraranas and rejected the subgenera of *Pristimantis* proposed by Hedges *et al*. (2008) because these authors observed paraphyly of *Pristimantis* with respect to *Hypodictyion* and reformulated the species series and species groups within *Pristimantis* of previous authors to make them explicitly monophyletic. Further, they also recognized Brachycephaloidea as a phenotypically diagnosable superfamily with the informal, unranked named terrarana retained as the common name for these frogs, Brachycephaloidea is a monophyletic clade recognized for seven morphological synapomorphies in the urogentital and vascular (Taboada *et al*., 2013) and it is characterized by having terrestrial breeding and direct development. Three families are recognized within Brachycephaloidea: Brachycephalidae, Craugastoridae and Eleutherodactylidae, the genus *Pristimantis* is allocated into the Craugastoridae.

***Pristimantis conspicillatus* group**

Lynch (1986) recognized the *Eleutherodactylus conspicillatus* group [=*Pristimantis conspicillatus* group] to accommodate those species once assigned to the *E. fitzingeri* group [=*Craugastor fitzingeri* group] (Lynch, 1976; Lynch & Myers, 1983) that are not members of the genus *Craugastor* which feature the "S" condition of the *m. adductor mandibulae*. This action left Lynch (1986) uncertain whether or not to propose a group defined by a set of plesiomorphic features. Latter, Lynch (1994) found a derived character that could separate species of the *P. conspicillatus* group from species of the *Craugastor* genus: the relative lengths of toes III and V.

Lynch & Duellman (1997) defined the *E. conspicillatus* group with the following features. "Frogs small to large (SVL in females 25-75 mm), with narrow heads and long snouts; cranial crest absent; upper eyelid usually narrow than IOD, rarely as wide as, or wider than, IOD; body slender; limbs relatively long; skin of venter smooth; vomerine odontophores large, triangular in outline, narrowly separated medially; vocal slits usually present; nuptial pads usually present; tympanic membrane and annulus present, relatively large, not sexually dimorphic in size.

Discs on fingers and toes expanded, those on inner fingers narrow; Finger I usually longer than finger II; subarticular tubercle not projecting; supernumerary tubercles few or absent; tarsus bearing short fold on inner edge; Toe V slightly longer than toe III, toes unwebbed or with basal webbing". Lynch & Duellman (1997), allocated 34 species into the *E. conspicillatus* group that occur in the lowlands of Costa Rica to southern Brazil and Bolivia and also inhabit the highlands of the Sierra Nevada de Santa Marta (SNSM) and Andes in Colombia, Ecuador and Peru.

The genus *Pristimantis* was subdivided in several species groups and series by Hedges *et al.* (2008), the former *E. conspicillatus* group is now *Pristimantis conspicillatus* group, this group contains most of the species recognized in the "*E conspicillatus*" group by Lynch & Duellman (1997). Hedges *et al.* (2008) redefined the content of the *E. conspicillatus* group proposed by Lynch (1986, 1994) and Lynch & Duellman (1997), and allocated 37 species to the group and showed support for the monophyly of this species group. Of the 37 species recognized in the *P. conspicillatus* group by Hedges *et al.* (2008) only ten are in the molecular phylogeny. This topology showed that *Pristimantis malkini* is sister species of *P. citriogaster* and these two species were clustered with *P. conspicillatus* and other species recognized as *Pristimantis sp* (=SBH266049). The other pair of sister species was *P. skydmainos* and *P. bipunctatus*. Five species previously assigned to *P. conspicillatus* group by morphology *sensu* Lynch & Duellman (1980) where removed from this group based on molecular information: *Pristimantis actites*, *P. w-nigrum* and *P. lanthanites* and these three species were cluster together into the *Pristimantis (Hypodictyon) rubicundus* group *sensu* Hedges *et al.* (2008). *Pristimantis caprifer* and *Pristimantis zeuctotylus* were not part of the clade in the molecular phylogeny; however, Hedges *et al.* (2008) retained these two species within the *P. conspicillatus* group.

One of the most significant changes proposed by Hedges *et al.* (2008) was to remove the species *Pristimantis peruvianus* from the *P. conspicillatus* group to be part of the new *P. peruvianus* group, this new group is a sister clade of *P. conspicillatus* group. *Pristimantis conspicillatus* and *P. peruvianus* were species previously considered to be only weakly separable and possibly conspecific (Lynch, 1975). Latter, Lynch (1980) removed the species *P. peruvianus* from the synonymy of *P. conspicillatus*.

Hedges *et al*. (2008) defined the *P. conspicillatus* group with the following features. "Frogs in this group are moderate to large in size with proportionately long hind limbs; head width is 30–43% SVL, and shank length is 45–64% SVL. Cranial crests are absent. The tympanic membrane and annulus are distinct. The dorsum is smooth or shagreen; a dorsolateral fold is present or absent. The venter usually is smooth, but it is weakly granular (aerolate) in some species. The toes commonly have basal webbing, and Toe V is only slightly longer than Toe III; an inner tarsal fold is present or absent. Lateral fringes are present or absent on the fingers and toes. Vocal slits usually are present. A dark facemask is apparent in many species; the most common dorsal color pattern consists of two or three chevron-shaped marks on the back".

Padial & De la Riva (2009) recognized 17 species on the lowlands east of the Andes in the *P. conspicillatus* group, including: *Pristimantis crepitans* (Bokermann 1965), *P. dundeei* (Heyer & Muñoz 1999), and *P. peruvianus* (Melin 1941); these had been removed from the group, and placed in the *P. peruvianus* species group by Hedges *et al.* (2008). *Pristimantis crepitans* and *P. dundeei* were the only species in the group known to inhabit the Cerrado. However, based on molecular data *P. crepitans* is now assigned to the genus *Oreobates* by Padial *et al.* (2012).

Pinto-Sánchez *et al.* (2012) showed that *Pristimantis achatinus*, *P. chiastonotus*, *P. gaigei* and *P. koehleri* clustered in *P. conspicillatus* group, increased the molecular phylogeny of *P. conspicillatus* group to fourteen species. In this topology, the two pairs of sister species observed by Hedges *et al.* (2008) were preserved: *P. malkini* with *P. citriogaster* and *P. skydmainos* and *P. bipunctatus*. Pinto-Sánchez *et al.* (2012) proposed *P. achatinus* and *P. lymani* as a sister species as well as *P. condor* with *P. conspicillatus*, and *P. fenestratus* with *P. koehleri*, the latter two species had already been discovered as cryptic species by Padial & De la Riva (2009). *Pristimanstis caprifer*, *P. lanthanites*, *P. actites*, *P. w-nigrum* and *P. zeuctotylus* also fall outside of the *P. conspicillatus* group. Pinto-Sánchez *et al.* (2012) included a sequence of *P. savagei;* species assigned to *P. conspicillatus* group by Lynch (1994) on the based on morphological information, but in this molecular phylogeny, *P. savagei* is clustered with *P. unistrigatus* species group. Finally, Pinto-Sánchez *et al.* (2012) rejected *P. conspicillatus* group monophyly and also showed that *P. peruvianus* group is a sister clade of *P. conspicillatus* group

Canedo & Haddad (2012) in order to test the monophyly of the genus *Ischnocnema* found that *Ischnocnema sensu* Hedges *et al.* (2008) was a polyphyletic with three named species *(I. paulodutrai*, *I. ramagii*, and *I. vinhai*) and two unnamed species recovered as part of the *P. conspicillatus* species group. These species are distributed in the Atlantic forest of Brazil, increasing the large and interesting distribution of the *P. conspicillatus* group. In this phylogeny also is observed that *P. peruvianus* group is a sister species of *P. conspicillatus* group.

Padial *et al.* (2014) in their molecular systematics of terraranas, found that *P. conspicillatus* group could be rendered monophyletic by making a few changes. The *P. conspicillatus* group molecular phylogeny increased to fifteen species, and based on molecular information confirms that *P. adiastolus* was part of the *P. conspicillatus* group. Again *P. malkini* with *P. citriogaster*, and *P. conspicillatus* with *P. condor* were conserved as a sister species. However, *P. koehleri* recognized as cryptic species of *P. fenestratus* by Padial & De la Riva (2009) forms a politomy with *P. fenestratus*, *P. chiastonotus* and *P. samaipatae* (Padial *et al.*, 2014).

Similarly, *P. skydmainos* and *P. bipunctatus* were recognized as sister species and these were clustered with *P. adiastolus*. As noted by the all the previous studies on molecular phylogeny of terraranas, *P. caprifer*, *P. actites*, *P. lanthanites* and *P. w-nigrum* fall outside of the *P. conspicillatus* group. Padial *et al*. (2014) found that terminal identified as *P. peruvianus* by Hedges *et al*. (2008) as well as those used by Pinto-Sánchez *et al*. (2012) are actually *P. reichlei*, therefore they transfer *P. peruvianus* to the *P. conspicillatus* group and the former *P. peruvianus* group was named as a *P. danae* group where was placed *P. reichlei* and they included *P. peru*vianus within the *P. conspicillatus* group following Lynch & Duellman (1997).

The Amazonian species allocate in the *P. conspicillatus* group

Many of the *P. conspicillatus* species group occur in the low elevation rainforest in the upper Amazon Basin in Brazil, Colombia, Ecuador and Peru: *P. conspicillatus*, *P. buccinator*, *P. citriogaster*, *P. fenestratus*, *P. lanthanites*, *P. malkini*, *P. metabastes*, *P. peruvianus* and *P. vilarsi*. The species *P. conspicillatus* and *P. peruvianus* were previously considered to be conspecific by Lynch (1975). These two species are weakly separable in size (*P. conspicillatus* is larger than *P. peruviaus)* and in the coloration pattern. In almost all the studies of molecular systematics of terraranas, *P. conspicillatus* is more closely related to *P. condor*, except for the study of Hedges *et al*. (2008) where *P. conspicillatus* is more related to *Pristimantis sp* SBH266049 (this species was only included by Hedges *et al.* (2008) in their study of maximum likelihood phylogeny of 216 species of frogs). So far, it has not been obtained sequence a *P. peruvianus* in order to determine how close it is to *P. conspicillatus*. The ranges of the two species broadly overlap in northeastern Peru, but they have not been found in sympatry, although both species have been collected in the vicinity of Tingo María, Departamento de Huánuco. *Pristimantis conspicillatus* apparently is confined to the lowlands, whereas *P. peruvianus* ascends the Amazonian slopes of the Andes to elevations of 1910 m in the Cordillera Oriental in Peru (Lynch & Duellman,1980), 1700-1975 m in the Cordillera de Cutucú (Duellman & Lynch, 1988), 1500-1830 m on the western slopes of the Cordillera del Cóndor (Almendáriz, 1997; Lynch & Duellman, 1980), 665-1750 m on the eastern slopes of the Cordillera del Cóndor (Reynolds & Icochea, 1997), 690-1280 m in the Serranía de Sira (Duellman &Toft, 1979), and 950-1080 m on the eastern slopes of the Cordillera Central in northern Peru (Duellman & Pramuk, 1999). Unpublished data suggest that *P. conspicillatus* contains numerous cryptic species therefore suggest a revaluation of its condition is necessary (Camacho-Badani *et al.*, 2013). Possibly other cryptic species are also now included in *P. peruvianus*. For example, there is a noticeable difference in size between individuals from elevations of 1410-1740 m on the Amazonian slopes of the Cordillera Oriental in Ecuador and those from the Amazonian lowlands, especially from Departamento Madre de Dios, Peru (Duellman & Lehr, 2009).

Pristimantis buccinator is characterized by having a pink coloration on groin and adjacent thigh and the presence of prominent dorsolateral folds. Padial *et al.* (2009) produced sequences for *P. buccinator* but this sequence has not been included in any other phylogenetic analysis and there is no relationship hypothesis for this species. The specific name of *P. citriogaster* is derived from the Greek kitrinos meaning citron yellow and the Greek gaster-meaning belly and alludes to the bright citron yellow color of the belly in life (Duellman, 1992). In all the molecular phylogeny of terrarana, *P. citriogaster* is the sister specie of *P. malkini* (Hedges *et al.*, 2009; Pinto *et al.,* 2012; Camacho-Badani *et al.*, 2013; Padial *et al.*, 2014*). Pristimantis malkini* is the only species allocated in the *P. conspicillatus* group that having an appreciable webbing of the toes and inner edge of the tarsus bearing a flaplike fold (Lynch, 1980). *Pristimantis metabastes* is known from only two specimens, neither of which is accompanied by data on habitat or coloration. The catalogue number of the holotype (KU 186904) given by Duellman & Pramuk (1999) is incorrect; the correct number is KU 196904. The specific name is a Greek noun meaning leaper. Duellman & Pramuk (1999) applied this name to this long-legged species with the pre-sumption that it is capable of lengthy jumps. There is no relationship hypothesis for this species. *Pristimantis lanthanites* is characterized by having a heel elongate and conical tubercle, the only other species of the *P. conspicillatus* group having heel tubercle is *P. thectopternus*. However, Lynch (1975) suggest that *P. lanthanites* is most closely related to *P. fenestratus* and *P. vilarsi* in having large digital pads, weakly tuberculate skin, similar head shape and profile, and uniform brown posterior surfaces of the thighs. Latter, Lynch & Suarez-Mayorga (2000) suggest that *P. lanthanites* is most closely related to *P. epacrus*, *P. fallax* and P. *gutturalis* in having supra anal warts. Based on molecular information, *P. lanthanites* is removed from the *P. conspicillatus* group and is allocated in the *P. rubicundus* group *sensu* Padial *et al*. (2014).

Pristimantis fenestratus is one of the most widely distributed species in the Amazon basin (100-1800 m), reported in eastern Peru, northeastern Bolivia, southeastern Ecuador, southeastern Colombia, and Brazil (Frost, 2015). Duellman & Lehr (2009) suggest that populations in Central Brazil represent different species than those populations near the base of the Andes in Peru and Bolivia. Padial & De la Riva (2009) describe *P. koehleri* as a sibling species of *P. fenestratus* that inhabitas the Amazonian and semidecidous forest of the Andean foothills in Central Bolivia. Melin (1941) provided the original description of *P. vilarsi*. Lynch (1975) provided a brief diagnosis, and commented on the coloration in preservative of few specimens, he noted that the concealed surfaces of the thighs are brown, the dorsum has extensive brown mottling, and transverse bars are present on the limbs. Barrio-Amorós & Molina (2006) noted three-color pattern in Amazonian Venezuela and determined that these are not significant geographically, and can be found in a same population from anywhere and concluded that in view that this huge variability, it is untestable that many names has been used for this single species, which is difficult to diagnose. Furthermore, these authors suggest that *P. zeuctotylus* cannot be readily distinguished from *P. vilarsi*, except for the round palmar tubercle and the absence of tarsal fold. Barrio-Amorós & Molina (2006) also showed an interspecific variation regarding the palmar tubercle shape (bifid to heart-shape to round) in *P. vilarsi.* Barrio-Amorós & Molina (2006) considered that the only differences between *P. vilarsi* and *P. zeuctotylus* is the presence of tarsal fold, therefore they wondered if the presence/absence of the tarsal fold is a sufficient to distinguish between two species that can be occur in sympatry. They suggest, if would not be better to suggest that *P. zeuctotylus* as synonym of *P. vilarsi*. At the moment, there are no sequences available for *P. vilarsi*.

Another species that has an Amazonian distribution is *P. skydmainos* which is distributed in Amazonian lowlands in central and southern Peru, and extreme western Brazil, and on the lower Amazonian slopes (up to 750 m) of the Cordillera Oriental in Peru and Ecuador (Frost, 2015). *Pristimantis skydmainos* superficially resembles *P. conspicillatus*, *P. peruvianus* and *P. fenestratus*. However, only *P. skydmainos* has an interocular fold (Flores & Rodriguez, 1997). In most the molecular phylogeny of terraranas, *P. skydmainos* and *P. bipunctatus* are sister species.

The Peruvian species allocated to the *P. conspicillatus*

Other species allocated to the *P. conspicillatus* group occur in Peru: *P. adiastolus*, *P. avicoporum*, *P. bipunctatus*, *P. meridionalis*, *P. nebulous* and *P. phalaroinguinis Pristimantis adiastolus* is distributed on the eastern slopes of the Cordillera Yanachaga in Departamento Pasco, central Peru (Frost, 2015). In the original publication, *P. adiastolus* is placed into the *P. peruvianus* group but in a phylogenetic tree based on molecular data on sequences of mitochondrial 12S rRNA gene showed by Duellman & Hedges (2005), *P. adiastolus* is clustered with *P. bipunctatus* (*P. conspicillatus* group). Duellman

& Hedges (2007) suggested that *P. adiastolus* and *P. bipunctatus* are cryptic species that have similarities in structure (weakly aerolate belly, long snout, and long legs) and coloration (pattern of dorsal chevrons, back scapular spots, and brown mottling on the throat). Nevertheless, *P. adiastolus* has prominent dorsolateral folds (low and barely discernable in *P. bipunctatus*, Finger I slightly shorter than Finger II (Finger II and I equal in length in *P. bipunctatus*), and distinctly shorter digits (Duellman & Hedges, 2007). Padial *et al.* (2014) showed that P. *bipunctatus* and *P. skydmainos* are sister species. *Pristimantis bipunctatus* is widely distributed in lowlands and cloud forest in central Peru (Frost, 2015). *Pristimantis phalaroinguinis* occurs in the Cordillera Occidental in northern Peru. Duellman & Lehr (2007) allocated this species to the *P. conspicillatus* group, having Finger I shorter than Finger II. There is no relationship hypothesis for this species. *Pristimantis avicoporum* and *P. meridionalis* are known only from the region of the type localities. *Pristimantis avicoporum* is from the Province of Bagua, Department of Amazonas whereas *P. meridionalis* is from Caraz, Department of Ancash. For these two species there are no available sequences. Lehr & Duellman (2007) suggested that *P. meridionalis* is more similar with *P. skydmainos* and *P. avicoporum* due to these three species sharing dark brown middorsal tubercle. *Pristimantis nebulous* is known only from the type locality, Huánuco. Lynch & Duellman (1997) placed *P. nebulous* into the *P. conspicillatus* group but it is unassigned to species group by Hedges *et al.* (2008) and Padial *et al.* (2014). Duellman & Lehr (2009) suggest that because the holotype is in bad condition association with a living population is problematic.

Pristimantis lymani occurs in semiarid valley of the Chinchipe and Huancabamba river drainages in department of Cajamarca, Piura and Lambayeque and north into the semiarid and mesic valleys of the Catamayo and Zamora rivers in Ecuador, and is found in sympatry with P. *phalaroinguinis.* Lynch & Duellman (1997) suggest that *P. lymani* is most closely related to *P. citriogaster* and *P. condor*, species found in the Amazonian, rather than the Pacific drainage. In most of the terrarana molecular studies *P. lymani* is the sister species of *P. achatinus*. *Pristimantis condor* occurs in the Cordillera del Condor and adjacent Amazonas, Peru and the Cordillera de Cucutú in Ecuador (Frost, 2015). Lynch & Duellman (1980) suggest that *P. condor* in most superficially similar (in preservative) to the Amazonian slopes populations of the smaller *P. peruvianus*, but has less intense brown marking on the throat and chest and narrower head. In most of the studies of molecular systematics of terraranas, *P. condor* is more closely related to *P. conspicillatus.*

Inter Andean dry valleys species of Bolivia allocated in the *P. conspicillatus* group

Pristimantis koehleri and P. samaipatae are species placed in the *P. conspicillatus* group that occur in Bolivia. *Pristimantis koehleri* is a sister species of *P. fenestratus* (Padial & De la Riva, 2009), these authors also argued that the origin of *P. koehleri* seems to be related to habitat condition different from those occupied by its sister taxa. As a mentioned above, *P. fenestratus* occurs in humid lowland Amazonian forest and humid forest of the Andean hills while *P. koehleri* inhabits the semi-deciduous forest of the Andes hills, at the southern edge of the distribution of *P. fenestratus*, both species share a small area of overlap along the humid Andean slopes of Central Bolivia. Padial & De la Riva (2009) recognized *P. koehleri* as cryptic species of *P. fenestratus* but in the last molecular phylogeny of terrarana, these two species form a politomy with *P. chiastonotus* and *P. samaipatae* (Padial *et al.*, 2014). *Pristimantis samaipatae* occurs in open forest of temperate inter-Andean valley of Santa Cruz and Tarija and adjacent to Argentina, sharing some localities with *P. koehleri.*

Cerrado Brazilian species allocated in *P. conspicillatus* group

Pristimantis dundeei and *P. ventrigranulosus* are species that occur in the Brazilian Cerrado. Heyer & Muñoz (1999) described *P. dundeei* differentiating it from *P. fenestratus* in advertisement call and morphology, although these authors did not assign *P. dundeei* within any *Pristimantis* group they argued similarities of *P. dundeei* with *P. fenestratus* (*P. conspicillatus* group). The belly of *P. fenestratus* is either entirely smooth or weakly aerolate only on the lateral most portions; the belly of *P. dundeei* is weakly aerolate at least posteriorly. Males of *P. dundeei* have two weakly developed light glandular nuptial asperities on each thumb; male *P. fenestratus* have a single, better-developed light glandular nuptial asperity in each thumb. Hedges *et al.* (2008) placed *P. dundeei* into the *P. peruvianus* group. Siqueira *et al.* (2008) in their study of chromosomal analysis of three Brazilian "eleutherodactyline" frogs suggest that *P. dundeei* may be in the *P. conspicillatus* group and that, more than one species is covered by this name and that the population from Aripuanã is unnamed. Padial & De la Riva (2009), named this population as *P. koehleri* and noted that the reports of *P. dundeei* from central Bolivia by Kohler *et al.* (2000) was also based on *P. koehleri*. Maciel *et al.* (2012) in the original description of *P. ventrigranulosus*, showed that this species has an advertisement call equal in structure to the calls of *P. dundeei*, *P. fenestratus*, *P. koehleri* and *P. vilarsi*; therefore, they transfer *P. dundeei* to the *P. conspicillatus* group.

Atlantic forest species allocated in *P. conspicillatus* group

Pristimantis paulodutrai, *P. ramagii* and *P. vinhai* are distributed in the Atlantic forest in Brazil. Lynch & Duellman (1997) placed *P. paulodutrai* and *P. ramagii* to the "*Eleutherodactylus ramagii*" group, while Lynch & Duellman (1997) left *P. vinhai* as an unassigned species group. Hedges *et al.* (2008)

allocated *P. paulodutrai* and *P. ramagii* into the *Ischnocnema ramagii* species series while *P. vinhai* was assigned into the *I. guentheri* species series. Canedo & Haddad (2012) found that three species attributed to *Ischnocnema* were clustered within the *P. conspicillatus* group and proposed a new combination for these three species and suggested that these may represent a species complex.

Pristimantis charlottevillensis occur in northeastern Tobago Island, Trinidad and Tobago, and was confused with *P. terraeboliviaris* by Murphy (1997). *Pristimantis terraeboliviaris* is distributed in the coastal range of Venezuela. Kaiser *et al.* (1995) found that *P. charlottevillensis* and *P. terraeboliviaris* have a great similarity at the morphological and chromosomal level may be an indicative a close phylogenetic relationship, and may be putative sister species. These species are readily distinguished from each other by their vocalizations, morphological details (hands and feet) or morphometrics, although these two last descriptive and qualitative methods are impractical when only a few specimens are available (Kaiser *et al.*, 1995).

Guiana species allocated in *P. conspicillatus* group

Pristimantis chiastonotus, P. gutturalis and *P. zeuctotylus* are known from northeastern South America from extreme southeastern Colombia through Amazonian Venezuela, French Guiana and Surinam, as well as scattered records from northern Brazil and southwestern Brazil. Padial *et al.* (2014) found that the terminal used by Hedges *et al.* (2008) and Pinto-Sánchez *et al.* (2012) as *P. zeuctotylus* was misidentified. Canedo & Haddad (2012) used another voucher and this was clustered with species of *P. conspicillatus* group. Lynch & Suarez-Mayorga (2000) suggested the only hypothesis of the relationships for *P. gutturalis*, where the conical supra anal warts represent a synapomorphies for the species: *P. epacrus*, *P. fallax, P. gutturalis* and *P.*

lanthanites. Although, *P. gutturalis* have sequences available, these have not been taken into account in a phylogenetic analysis of terrarana. Similarly, there is no hypothesis of relationship for *P. chiastonotus*; this species forms a politomy with *P. fenestratus, P. koehleri* and *P. samaipatae*. Padial *et al.* (2014) recognized these three species in the *P. conspicillatus* group.

Northern Andes species allocated in *P. conspicillatus* group

Pristimantis actites, *P. caprifer* and *P. illotus* occur on the cloud forest of the Pacific slopes of the Andes in Colombia southward to northwestern and central Ecuador. *Pristimantis actites* is an endemic species of the Cordillera Occidental in Ecuador. Lynch & Duellman (1997) suggested that *P. actites* is most closely to *P. w-nigrum*, these two species are very similar in external morphology. In all terrarana molecular phylogenies, *P. actites* is the sister species of *P. w-nigrum*, and in all the topologies they are not members of the *P. conspicillatus* group, instead of these two species are allocated in the *Pristimantis ridens* group *sensu* Padial *et al.* (2014). Lynch & Duellman (1997) considered that *P. actites* is the geographic replacement of *P. w-nigrum*, which occurs at both higher and lower elevations to the north and to the south of Pilaló and, thus, has a range encompassing that of *P. actites*. This hypothesis is based is on the observations that these two species have the same microhabitat, phenetic similarity and the failure to find these two species in sympatry.

Lynch & Duellman (1997) followed Lynch & Myers (1983) assigned *P. caprifer* to *P. conspicillatus* group, although it has some characteristics that do not conform to the *P. conspicillatus* group. *Pristimantis caprifer* has the skin of the dorsum smooth and the venter aerolate, and Finger I is shorter than Finger II. In all the molecular studies *P. caprifer* falls outside of the *P. conspicillatus* group and falls in different parts of the tree inside the genus *Pristimantis*, and for this reason is not assignable to a species group according to Padial *et al.* (2014).

Lynch & Duellman (1997) suspected that *P. illotus* and *P. thectopternus* are sister species but they are unaware of any synapomorphy to link these two species. There is currently no molecular information available for these two species. Padial *et al.* (2014) leave *P. illotus* unassigned to any *Pristimantis* species group.

Pristimantis achatinus is distributed from extreme eastern Panama south through the Chocoan lowlands of Colombia to southern western Ecuador and in the river valley of Cuaca in Colombia. Lynch & Myers (1983) were concerned that they might have combined distinct species that differed at least in part by frequency of two patterns polymorphism. Their concern was heightened because tape recording of frogs from Panama ("ribit "calls) are notably different from a recording of *P. achatinus* from Ecuador ("kree" call), and therefore Lynch & Duellman (1997) suggested that *P. achatinus* is a complex of cryptic species that have different calls, but that are structurally so similar that is difficult to distinguish more than one species. In all the molecular studies *P. achatinus* is the sister species of *P. lymani*. However, none of these studies has sequences from different locations in order to determine whether *P. achatinus* is actually a complex of cryptic species.

Pristimantis gaigei also occurs in Colombia on the Pacific versant in central Colombia as well as populations in the river valley of the Sinu, Cauca and Magdalena. *Pristimantis gaigei* is distributed from Atlantic drainages lowlands from extreme southeastern Costa Rica to eastern Panama and Colombia. *Pristimantis gaigei* was assigned to *P. conspicillatus* group despite having little resemblance to other species in the group (Lynch, 1980; Lynch & Duellman, 1997). This species has a first finger much longer than in other species of the *P. conspicillatus* species group, round and extremely large tympanum and prominent subarticular tubercles.

Pristimantis thectopternus and *P. w-nigrum* are species that may be within the distribution of any of the species mentioned above. Further, *P. thectopternus* and *P. w-nigrum* have been collected sympatrically. *Pristimantis thectopternus* occurs in the northern half of Cordilleras Central and Occidental at moderate elevations. *Pristimantis thectopternus* is a moderate sized frog that is characterized by having an elongate calcar, *P. lanthanites* is another species assigned to *P. conspicillatus* group that has a calcar. However, this calcar has not been taken into account for a hypothesized relationship. Lynch & Duellman (1997) suggest that *P. thectopternus* is most closely to *P. illotus*. Padial *et al.* (2014) removed *P. thectopternus* from the *P. conspicillatus* group and placed it in the *P. ridens* group without any argument or data.

Pristimantis w-nigrum is probably the species with the widest distribution range assigned to the *P. conspicillatus* group, this species occurs in the lower montane and cloud forest at 800-3000 m elevation on both Pacific and Amazonian slopes of the Andes in Colombia, Ecuador and extreme northern Peru (Frost, 2015). In populations of Ecuador, Kieswetler & Schneider (2013) reported on molecular phylogeography and found deep divergences that are geographically significant; implying that under the name *P. w-nigrum* may be more than one species. In all terrarana molecular phylogenies, *P. w-nigrum* is the sister species of *P. actites,* and in all the topologies they are not members of the *P. conspicillatus* group instead, these two species are allocated in the *Pristimantis ridens* group *sensu* Padial *et al.* (2014).

Other species such as a *P. carlossanchezi*, *P. carranguerorum*, *P. epacrus*, *P. ixalus*, *P. medemi*, *P. padrecarlosi* and *P. savagei* are distributed on the Andean slopes of the Cordillera Oriental of Colombia. None of these species has molecular hypothesis relationship except for *P. savagei*. This last species according to Pinto-Sánchez *et al.* (2012) is not assignable to *P. conspicillatus* group; in their study *P. savagei* clustered within *P. unistrigatus* group species. Padial *et al.* (2014) also excluded *P. savagei* from the *P. conspicillatus* group following Pinto-Sánchez *et al.* (2012) and, left this species unassigned to any *Pristimantis* species group. Lynch (1994) suggested that *P. carranguerorum* is most similar to *P. savagei* but differs in being larger, in lacking enlarged tubercles on the upper eyelid and heel, and in having a less acuminate snout, also Lynch (1994) argued that these two species on the eastern slopes of the Cordillera Oriental appear to replace one another. Likewise, Lynch (1994) suggested that *P. medemi* may be the sister species of *P. vilarsi* but is easily distinguished from it by its longer legs and smaller tympana. *Pristimantis ixalus* is known only from the type locality in the northern Cordillera Oriental (Departamento de Santander). Lynch (2003) suggested that *P. ixalus* is most similar to *P. w-nigrum*, from which it differs in having a longest snout, heel and outer tarsal tubercle, absence of vocal slits. *Pristimantis padrecarlosi*, also occurs in the northern Cordillera Oriental. Muses-Cisneros (2006) suggested that this species is similar to *P. carranguerorum* and *P. savagei*. Another species that occurs on the western slope of the Cordillera Oriental in the Departamento de Santander and might be sympatric with *P. padrecarlosi* is *P. carlossanchezi*. Arroyo (2007) placed *P. carlossanchezi* into the *P. conspicillatus* group given its morphological similarities with *P. carranguerorum*. Padial *et al.* (2014) removed *P. padrecarlosi* and *P. carlossanchezi* from the *P. conspicillatus* group and left these unassigned to species group. *Pristimantis pedimontanus* occurs along the southern versant of the Cordillera de Merida from the state of Táchira to the state of Lara, in northwestern Venezuela. La Marca (2004) in the original publication allocated this species in the *P. conspicillatus* group and suggested that this species is most similar with *P. carranguerorum*. Barrio-Amorós & Molina (2006) argued that *P. pedimontanus* does not have the morphological characteristics of the *P. conspicillatus* species group, and was removed from the *P. conspicillatus* group and left unassigned to species group by Padial *et al.* (2014). At the moment, there are no sequences available for *P. pedimontanus*.

Pristimantis epacrus is from the southern part of the Cordillera Oriental of Colombia. Lynch & Suarez-Mayorga (2000) considered that *P. epacrus* is most closely related to *P. fallax*, *P. gutturalis* and *P. lanthanites* with which it shares conical supra anal warts. These conical supra-anal warts have been hypothesized as a synapomorphy linking these four species in the *P. conspicillatus* group. Padial *et al.* (2014) allocated *P. carranguerorum* and *P. medemi* to the *P. conspicillatus* group, while *P. epacrus* and *P. ixalus* were assigned in the *P. ridens* group.

Pristimantis fallax, P. johannesdei, and *P. viridicans* are other species assigned to *P. conspicillatus* group that are distributed only in Colombia. None of these species has molecular information available. *Pristimantis fallax* occurs along forested streams on the eastern flank of the Cordillera Central. Lynch (1999) considered that *P. gutturalis* closely resembles *P. fallax* for its throat pattern (narrow raphe) but has larger warts scattered over the shagreen dorsum and lacks fringes on the toes. *Pristimantis johannesdei* occurs in the western flank of the Cordillera Occidental. According with the original publication, this species is similar to *P. achatinus* (Rivero & Serna, 1988), but differs in having the first finger shorter than second, absence of dorsolateral folds and in color pattern. *Pristimantis viridicans* occurs in the western flank of the Cordillera Occidental of Colombia in the departments of Valle del Cauca and Cauca. The specific name of *P. viridicans* meaning becoming green, in reference to the color of life, is grayish tan with cream cast to light green, with brown to black markings. Lynch (1977) considered *P. viridicans* most similar to *P. insignitus*, both have weak frontoparietal depression (low cranial crest), narrow digital pads, and short, obscure inner tarsal folds. Padial *et al.* (2014) left *P. fallax* and *P. viridicans* unassigned to any *Pristimantis* species group while *P. johannesdei* remained in the *P. conspicillatus* group.

Pristimantis carmelitae and *P. insignitus* inhabit the highlands of the Sierra Nevada de Santa Marta. Lynch & Ruiz-Carranza (1985) were undecided about where to place *P. carmelitae*, because this species has the first finger shorter than second and in having depressed tympana, subsequently placed into the *P. conspicillatus* group by Lynch (1994) and removed from the *P. conspicillatus* species group and left unassigned to species group by Padial *et al.* (2014).

Although molecular data provide evidence of the monophyly of *P. conspicillatus* group, many questions remain open within the *Pristimantis conspicillatus* group. Herein, we are interested in knowing the evolutionary relationship within *P. conspicillatus* group and gain a better understading of the alfa diversity of this group. In the chapter 2, we inferred the phylogenetic relationships of the *P. conspicillatus* group including all the species that in different scenarios and with different data (molecular and morphological) have been considered as a member of *P. conspicillatus* group (Table 1).

Table 1. Species *assigned to the Pristimantis conspicillatus* group

Species	**Lynch & Duellman (1997)**	**Hedges *et al.* (2008)**	**Padial *et al.*(2014)**
P. achatinus (Boulenger, 1898)	x	x	X
P. adiastolus (Duellman & Hedges, 2007)		x	X
P. avicuporum (Duellman & Pramuk, 1999)		x	X
P. bipunctatus (Duellman & Hedges, 2005)		x	X

Species	**Lynch & Duellman (1997)**	**Hedges *et al.* (2008)**	**Padial *et al.*(2014)**
P. buccinator (Rodríguez, 1994)	x	x	X
P. caprifer (Lynch, 1977)	x	x	
P. carlossanchezi (Arroyo, 2007)		x	X
P. carmelitae (Ruthven, 1912)	x	x	
P. carranguerorum (Lynch, 1994)	x	x	
P. charlottevillensis (Kaiser, Dwyer, Feichtinger & Schmid, 1995)		x	X
P. chiastonotus (Lynch & Hoogmoed, 1977)	x	x	X
P. citriogaster (Duellman, 1992)	x	x	X
P. condor (Lynch & Duellman, 1980)	x	x	X
P. conspicillatus (Günther, 1858)	x	x	X
P. dundeei (Heyer & Muñoz, 1999) *			X
P. epacrus (Lynch & Suárez-Mayorga, 2000) *			
P. fallax (Lynch & Rueda-Almonacid, 1999) *			
P. fenestratus (Steindachner, 1864)	x	x	X
P. gaigei (Dunn, 1931)	x	x	X
P. gutturalis (Hoogmoed, Lynch & Lescure, 1977)	x	x	X
P. illotus (Lynch & Duellman, 1997)	x	x	

Species	**Lynch & Duellman (1997)**	**Hedges *et al.* (2008)**	**Padial *et al.*(2014)**
P. insignitus (Ruthven, 1917)	x	x	
P. ixalus (Lynch, 2003)*			
P. johannesdei (Rivero & Serna, 1988)	x	x	X
P. koehleri (Padial & De la Riva, 2009)*			X
P. lanthanites (Lynch, 1975)	x		
P. lymani (Barbour & Noble, 1920)	x	x	X
P. malkini (Lynch, 1980)	x	x	X
P. medemi (Lynch, 1984)	x	x	X
P. meridionalis (Lehr & Duellman, 2007)			X
P. metabates (Duellman & Pramuk, 1999)			X
P. nebulosus (Henle, 1992)			
P. paulodutrai (Bokermann, 1974)			X
P. pedimontanus (La Marca, 2004)			
P. peruvianus (Melin, 1941)			X
P. phalaroinguinis (Duellman & Lehr, 2007)			X
P. ramagii (Boulunger, 1888)			X
P. samaipatae (Köhler & Jungfer, 1995)			X
P. savagei (Pyburn & Lynch, 1981)			
P. skydmainos (Flores & Rodríguez, 1997)			X

Species	**Lynch & Duellman (1997)**	**Hedges *et al.* (2008)**	**Padial *et al.*(2014)**
P. terraebolivaris (Rivero, 1961)			X
P. thectopternus (Lynch, 1975)	X		
P. ventrigranulosus (Maciel, Vaz-Silva, Oliveira, and Padial, 2012)*			
P. vilarsi (Melin, 1941)			X
P. vinhai (Bokermann, 1975)			X
P. viridicans (Lynch, 1977)	X		
P. w-nigrum (Boettger, 1892)	X		
P. zeuctotylus (Lynch & Hoogmoed, 1977)			X

A geological background hypothesis of the northern Andean formation

The northern of Andes extend from 5°S at about the Amotape Cross at 2°S to the Oca, Romeral, and other fauld systems across northern South America at the contact with the Caribbean Plate at 12°N (Graham, 2009). In Ecuador, the Andes cordillera extend in two main ranges roughly parallel to the oceanic trench, from the Amotape-Huacabamba depression, to the "Nudo de los Pastos" in south of Colombia, where the Andes branch into three ranges; an acreced arc (Western Cordillera), and two fold-thrust belts (Central and Eastern cordilleras) (Gregory-Wodzicki, 2000; Taboada & Rivera, 2000), the Eastern Cordillera branches again into the Western Sierra Nevada de Santa Marta and Serranía de Perijá in the colombo-venezuelan border, and the eastern Cordillera de Mérida in Venezuela (Graham 2009).

The northern Andes block is also divided based on lithotectonic and morphostructural data, not on present major physiographic features (Cediel *et al.*, 2003) as: a) Guiana Shield Realm (GSR), b) Central Continental Sub-Plate Realm (CCSP), c) Maracaibo Sub-Plate Realm (MSP), d) Western Tectonic Realm (WTR) and e) Guajira-Falcon Composite Terrane (GU-FA). According to Cediel *et al.* (2003) this kind of analysis avoids incurring the false notion that, for example, a certain cordillera or depression today corresponds to a single lithotectonic unit or represents a single geotectonic event. Even when the arguments of Cediel are convincing, most of the literature is based on the classic geological view.

The formation of an orogeny or group of mountain, involves two related but separate geological processes that frequently are misunderstood by non-specialists; on one hand, the exhumation process, that consists of the emergence of metamorphic rocks to the earth surface (Ring *et al.*, 2010), and on the other hand, the uplift, that can be separated between surface uplift, meaning the vertical motion of the earth surface respect to the sea level, and rock uplift, meaning the vertical upward motion of the rocks respect to the sea level, the differences between rock uplift and surface uplift are because of the erosion effect (England & Molnar, 1990). In general terms, much more evidence and high confidence information concerns exhumation than about uplift, and many reports about surface uplift are really about rock uplift or exhumation of rocks (England & Molnar, 1990). In the northern Andes many information gaps remain with respect to the time and rates of surface uplift, and much work remains to be done especially in the field of paleobiology (Montes, com. pers.).

The Ecuadorian Andes

The active continental margin of Ecuador is characterized by the subduction of the Nazca Plate below the South American Plate, at a mean rate of 58 mm/yr. The Western cordillera of Ecuador is composed of oceanic rocks accreced to the continent along a major suture zone, during the cretaceous. The Inter-Andean depression correspond to an allochthonous block characterized by an uppermost Pliocene-Pleistocene volcanic deposits, wish is located between two N-S trending basement faults. The Eastern cordillera of Ecuador (Cordillera Real) is a Paleozoic metamorphic belt, which overthrusts the sub-Andean zone located eastward, along the North Andean Frontal fault (Aspden & Litherland, 1992).

The Ecuadorian Cordillera was essentially lifted as a great horst, but when lifted beyond the height that block strength could bear, the horst started to spread laterally, forming divergent faults and an extensional graben (the Interandean Depression) in the middle. In early Miocene the Ecuadorian Cordilleras did not exist and the Pacific Ocean reached the Oriente, and, in the Middle Miocene, the uplift of an elongated swell, consisting of Palaeozoic and older rocks, created the Eastern Cordillera. The present mountain chain was created only during the Plio-Pleistocene (Coltorti & Ollier, 2000).

The Colombian Andes

The nature and composition of the three Colombian Cordilleras are substantially different, each one resulting from a distinct tectonic process that affect the north-west of South America during the Mesozoic and Cenozoic (Taboada & Rivera, 2000). The Romeral fault which extends along the boundary between the Cauca-Patia depression and the Central Cordillera, subdivides the Colombian Andes into the “Occidente” and “Oriente” regions (Taboada *et al.*, 2000). The Romeral fault is also the limit between the WTR and the CCSP (Cediel & Shaw, 2003) and extends to the south to the Ecuadorian suture.

The southern part of the Colombian Western Cordillera, that shares the Piñón-Daqua block with the Ecuadorian Western Cordillera, is formed by thrust and fold belt linked to the Nazca subduction. The northern part of the Colombian Western Cordillera, belong to the Chocó block and part of the Caribbean block. This part has been also influenced by the accretion of the Caribbean block (Taboada & Rivera, 2000; Cediel & Shaw, 2003). To the north-west of the Western Cordillera emerge the Baudó-Panamá ranges, product of the collision between the eastern part of the island arc and nortwestern South America, mainly during the late Miocene and Pliocene (Taboada & Rivera, 2000).

The time when the central part of the eastern Cordillera began the transformation between tropical to montane habitats, was possibly between 30 and 26 MA (Oligocene) and close to Bogotá, ca. 23 MA (latest Oligocene-early Miocene) in the north of Boyacá, and ca. 10 MA, on the western slopes close to Guaduas (Cundinamarca) (Ochoa *et al.*, 2012). This means that the process was not synchronous, and that most began long before the latest major uplift of the Eastern Cordillera. Additionally, Ochoa *et al.* (2012) concluded that by the early Miocene the basin of the Magdalena River was already separated from the Llanos basin, but this topographic barrier (the eastern cordillera), did not reach elevations higher than 1000 m. between the Oligocene and Late Miocene, appeared gradually montane elements in the palynoflora record. That evidence does not disagrees with Van der Hammen *et al.* (2013) who argued that the major uplift of the eastern cordillera was during the middle-late Pliocene. This conclusion is also supported by most recent and different sources of data, which put the main phase of topographic growth between 6 and 3 MA (Mora *et al.*, 2008).

The Venezuelan Andes

The northeastern Andes begin in the Miocene but reach a major phase of uplift between 5 and 3 Ma (Van der Hammen *et al.*, 1973). Particularly, the Merida Andes was formed at late Oligocene (33.9-23.03 Myr ago) (Kohn *et al.*, 1984). Meanwhile, the Santander Massif, in the limit between Colombia and Venezuela arose between early and middle Miocene, with an older uplift, particularly of the western part of 19-14 Ma *(*Kroonenberg *et al.*, 1990).

Andean diversification

The rise of the Andes is the principal cause of the great biodiversity in the Neotropics (Gentry, 1982; Richardson *et al.*, 2001; Jaramillo *et al.*, 2006). However, the evolutionary history of the biota on the different Andean biomes is fundamentally different (Särkinen, *et al.*, 2012a). It has been shown that these biomes, seasonally dry tropical forest (SDTF), mid-elevation montane forest (MMF) and high-elevation grassland (HEG) (Cuatrecasas, 1979), in addition to the compositional differences, reflect niche conservatism, and differences in species age and rate of diversification (Särkinen *et al.*, 2012a).

In northern Andes, the SDTF as the HEG have typical island distribution, however with contrasting ages of diversification, with the very oldest age in the SDTF. The HEG, that is fundamentally the Páramo habitat in the northern Andes, is a very new habitat, which includes an ecological radiation of species, with origin in the lower altitudinal ranges and temperate regions. The final pulse in the rise of the Andes during the Pliocene (Garzione *et al.*, 2008; Gregory-Wodzicki, 2000), as well as the Pleistocene glaciations, with the concomitant fall and rise of vegetation bands (Van der Hammen *et al.*, 1973) may have been the responsible the explanation for the principally quaternary age of the extent biota in the HEG.

The Andes have also created an east-west barrier to the dispersal of organisms, isolating previously separated populations (Lynch & Duellman, 1997; Pennington & Dick, 2010). The same system of mountains was a gate to the dispersion of temperate elements, mostly to the HEG (Hoorn *et al.*, 1995; Gengler-Nowak, 2002; Antonelli *et al.*, 2009). In fact, there are signals in different groups of plants and animals of high altitude, for colonization from higher latitudes, such as in Butterflies (Descimon, 1986). On the other hand, because the older age of the interandean valleys, some sister species inhabiting on isolated SDTF show very old times of divergence (Särkinen *et al.*, 2012b).

Even when it is generally accepted the importance of Andean uplift as a driver of diversification into the Andes and on contiguous lowlands (Gentry, 1982), there remain important questions about the timing and mode of diversification, and the relative importance of climatic change, especially the Quaternary glaciations, on diversification. With respect to the time, some debate has focused on the tertiary vs. quaternary importance for the formation of the modern biota of the Neotropics. According to paleobotanical evidence (Hooghiemstra & Van Der Hammen, 1998), Miocene/Pliocene plant diversity may have been greater than at present, and the modern phytodiversity may be more a product of the Tertiary rather than a product of the Quaternary. In that direction, (Jaramillo *et al.*, 2006), claim that many modern genera were extant and clearly recognizable in the pollen of Miocene sediments (23-6 Myr ago).

The theory of the Pleistocene refuges (Haffer, 1969), proposed environmental stability zones acting as islands, and promoting speciation during the

Pleistocene oscillation of global temperature. Even when this theory was originally proposed for Amazonia, and has been highly controversial (Bush & Oliveira, 2006), it is not yet clear what was the impact of the Pleistocene climate on the tropical Andean fauna. However, there are examples of groups from MMF whose species diversity comes mostly from the quaternary (Richardson *et al*., 2001) and several groups of the HEG, have radiated in the Plestocene (Hughes & Eastwood, 2006).

In term of the origin and mode of speciation of the Tropical Andean flora and fauna, hypothetically, a tropical montane fauna can evolve vertically, through upward or downward speciation, or horizontally through speciation between mountains, or from higher latitudes (Chapman, 1917; Lynch, 1986; Willmott *et al*., 2001), proposed two possible scenarios for speciation in the tropical Andes, one static onto existing mountains and vegetational zones, and other dynamic, governed by orogenic and vegetation changes.

Diversification in the Amazon

The extraordinary fauna and flora diversity of the Amazon basin has led to the formulation of a different historical hypothesis to explain the extraordinary species richness of the Amazon basin (Haffer, 1997; Moritz *et al*., 2000; Cheviron *et al*., 2005; Wüster *et al*., 2005). Various biogeographic mechanisms have been proposed to explain diversifcation in the Amazon, including allopatric speciation via riverine barriers (Wallace, 1852), forest refugia (Haffer, 1969, 1997), marine incursions (Nores, 1999; Webb, 1995), historic mountain ridges (Räsänan *et al*., 1990), or climatic disturbance (Colinvaux *et al*., 1996; Colinvaux, 1998), as well as parapatric speciation caused by divergent selection across ecological gradients (Endler, 1977). Recent advances in the fields of palynology, paleontology, climatology, and phylogenetics have provided new insights into the diversification of the organisms and environmental changes in lowland Amazonia (Hoorn *et al.*, 2010). For example, evidence of a dramatic change in the course of the Amazonian rivers during the late Pliocene was reported by Espurt *et al.* (2010), which was mediated by neotectonics following the establishment of the Fitzcarrald Arch (Fernandes *et al.*, 2013). This continental-wide drainage reorganization of the Amazon Basin is now assumed as the main driver of avian speciation in Amazonia (Ribas *et al.*, 2012).

Riverine barrier hypothesis (Wallace, 1852) suggests that widespread Amazonian organisms are thought to have split into isolated populations due to the formation of the Amazon River network (Antonelli *et al.*, 2010). Riverine barrier effects have been cited to explain distribution limits in a number of vertebrate species in the Amazon basin, including primates (Ayres & Clutton-Brock 1992) and birds (Bates *et al.*, 2004). These last authors in birds, found that meandering rivers arising in the basin might offer more opportunities for gene flow whereas faster flowing rivers arising in the Andes or the Guiana and Brazilian Shields might be stronger barriers. Nevertheless, studies in anurans and mammals (Lougheed *et al.,* 1999, Gascon *et al.*, 2000) have shown that at least some major rivers in Amazonia do not appear to have promoted the diversification of Amazonian animals. However, rivers appear to have played a role in population differentiation in some Amazon frogs, as demonstrated in a phylogeographic analysis of *Engystomops petersi* across three large rivers in western Amazon (Funk *et al.*, 2007).

Pleistocene refugia hypothesis (PRH) postulates that the area of Amazon forest was reduced during the dry periods due to global climatic fluctuations occurring throughout the late Pliocene and Pleistocene (Haffer, 1969; Vanzolini & Williams, 1970). The savanna and seasonally dry forests are assumed to have functioned as barriers separating populations of rain forest species, facilitating allopatric speciation (Haffer, 1969, 1997; Haffer & Prance, 2001). The main debate about the PRH has focused on the fact that palynological evidence provides little support for widespread savanna vegetation in Amazonia, at least during the last glacial maximum and late Pleistocene (the past 18,000–50,000 years) (Haberle & Maslin, 1999; Colinvaux *et al.*, 2000, 2001). Nonetheless, pollen records from the Early and Middle Pleistocene are lacking, hence, a hypothesis of rain forest fragmentation in the Middle or Early Pleistocene can be neither rejected nor confirmed by the palynological evidence currently available (Wüster *et al.*, 2005)

During the Miocene, global sea level fluctuations above the present-day level (Haq *et al.*, 1987; Miller *et al.*, 2005) periodically influenced large parts of lowland Amazonia (Hoorn & Vonhof 2006). Nores (1999) argued that during periods of marine incursions two large islands in northeastern South America would have existed around the Guiana Shield. Moreover, a large number of smaller islands and archipelagos would have been formed along the coastal lowlands of north eastern South America and the southern periphery of the Amazon drainage basin (Antonelli *et al*, 2010). Marine incursions entering the proto-Amazon drainage basin from the north could have divided populations into eastern vs western lineages during the Middle Miocene. In Amazon anurans, Noonan & Wray (2006) argued that such a spatial and temporal pattern can indeed be observed today in the phylogeny of Amazonian frogs, but that this pattern has been mistakenly attributed to ancient geological arches (Lougheed *et al.*, 1999; Symula *et al.*, 2003).

Andean speciation can contribute to Amazonian diversity. Santos *et al.* (2009) rejected an Amazonian center of origin in favor of a complex connectivity model expanding over the Neotropics. They inferred 14 dispersals nto and 18 out of Amazonia to adjacent regions, and argued that the Andes was the major source of dispersals into Amazonia.

There are few studies that consider the ancestral reconstruction of elevational distribution, because in some cases the study group is restricted to lowlands or to highlands, a poor taxonomic sampling or because the consideration of broad biogeographical regions. The few studies that considered the altitudinal factor have shown that the vertical diversification from lowlands to highlands is more common, and that allopatric speciation is most common in older divergence events, (Bates & Zink, 1994) in birds, (Hall 2005) in Butterflies. Less common are the cases of lowland species derived from higher altitudes, in contra the hypothesis of "species-pump", proposed for birds by Fjeldså (1994). This hypothesis invoked that the highland species of the Andes have acted as a species pump for lowland diversity and vice versa is based on the idea that in situ radiations in either highlands or lowlands eventually promoted multiple independent colonizations of adjacent areas at different elevations (Fjelds, 1994; Aleixo & Rossetti, 2007; Sedano & Burns, 2010).

In this study, we obtained a phylogeny of *P. conspicillatus* group, whose members are distributed in the lowlands and cloud forest from Costa Rica to Bolivia, across the Andean cordillera, Brazil (Amazonian, Cerrado and Atlantic Forest) and Guiana. In the chapter 3, we combined the phylogentic information, and biogeographic areas and elevational gradient in order to reconstruct the geographic and elevational radiation of *P. conspicillatus* group.

Chapter 2. Phylogeny and Character Evolution in the *P. conspicillatus* Group

Introduction

The genus *Pristimantis* Jiménez de la Espada, 1870, was resurrected for the clade including most former South American *Eleutherodactylus* by Heinicke *et al.* (2007) and Hedges *et al.* (2008) in their molecular study. The genus *Pristimantis* currently comprises 484 species (Frost, 2015), being the most diverse genus of terrestrial vertebrates. Its species are distributed through most of the Neotropics from Central America, throughout the North and Central Andes, Amazonas, Atlantic Forest Brazil and the Guyanas; Trinidad and Tobago; Granada and Lesser Antilles (Frost, 2015).

The monophyly of *Pristimantis* has been corroborated by subsequent analyses of molecular data (Pyron & Wiens, 2011; Canedo & Haddad, 2012; Pinto-Sánchez *et al.*, 2012; Padial *et al* 2014), however no morphological synapomorphy has been identified. Hedges *et al.* (2008) placed 16 species group in *Pristimantis*, including all groups previously recognized by Lynch & Duellman (1997), the former species group *Eleutherodactylus unistrigatus*, *E. conspicillatus* and 14 other species groups. Padial *et al.* (2014) rejected most of the groups proposed by Hedges *et al.* (2008) because they found them non monophyletic in their analysis and reformulated the species series and species group within *Pristimantis*. A few species groups were considered as a monophyletic, such the P. *conspicillatus* group.

Lynch (1986) recognized the former *Eleutherodactylus conspicillatus* group [=*Pristimantis conspicillatus* group] to accommodate those species assigned to the *E. fitzingeri* group [=*Craugastor fitzingeri* group] (Lynch, 1976; Lynch & Myers,

1983) that were not members of the subgenus *Craugastor* [= genus *Craugastor*, Crawford & Smith, 2005; Frost *et al.,* 2006; Hedges *et al.,* 2008]. The former species of the *E. conspicillatus* group closely resemble several of those in the subgenus *Craugastor* but *conspicillatus* species are distinguished by an external feature: these frogs have the fifth toe longer than the third, whereas frogs of the *fitzingeri* group have a fifth toe shorter than the third toe (Lynch, 1994; Lynch & Duellman, 1997). Additionally, both groups are differentiable by jaw musculature formulae (Lynch, 1986).

Lynch & Duellman (1997) recognized 34 species for the *P. conspicillatus* group. Members of this group—*sensu* Lynch (1994) and Lynch & Duellman (1997) occur in the lowlands of Costa Rica to southern Brazil and Bolivia and also inhabit the highlands of the Sierra Nevada de Santa Marta (northern Colombia) and Andes in Colombia, Ecuador and Peru. Hedges *et al.* (2008) redefined the content for the *P. conspicillatus* group proposed by Lynch (1986, 1994) and Lynch & Duellman (1997). They (Hedges *et al.* 2008) included 37 species in the *P. conspicillatus* group and found this group monophyletic. Padial *et al.* (2014) redefined its content allocating 33 species. Although molecular data provide evidence of the monophyly of *P. conspicillatus* group, the phylogenetic relationships within the *P. conspicillatus* group remain poorly understood. Moreover, this group lacks clear diagnostic characters and no phenotypic synapomorphies has been proposed so far.

The *Pristimantis conspicillatus* group, because of its remarkable species richness and distribution, provides a model for investigating the processes responsible for the generation of biodiversity and morphological innovation in the large genera. A prerequisite to understanding such processes is a reliable estimate of phylogenetic relationships. Our goals in this chapter are: 1) Test the monophyly of *P. conspicillatus* group and identify its sister group, 2) explore the relationships among its species, 3) determine the alpha diversity of *P. conspicillatus* group, 4) elucidate the potential number of unnamed species in the group on the basis of molecular data, and 5) explore some morphological characters observed in the *P. conspicillatus* group in the context of our phylogeny hypothesis.

Materials and Methods

Optimality criteria

Since the introduction of an explicit methodology for the inference of the phylogenetic relationships between organisms (Henning, 1965), there has been a long debate about the legitimacy of different approaches. These discrepancies increased in the last decades, with the implementation of molecular data in phylogenetic analysis. While in the past, the centre of the discussion was about issues as the search for criteria and methodologies to identify accurately the homology between phenotypical characters, today, the focus of debate is the correct interpretation of the molecular data for the inference of phylogenies, and the validity of derived applications of calibrating phylogenies that assume the molecular clock, such as the diversification analysis and reconstruction of ancestral area.

Methods of phylogeny reconstruction are divided into statistical methods (which require an explicit model of evolution) and non-statistical methods (Goloboff & Pol, 2005). Parsimony is a non-statistical, non-parametric, with a conservative approach to scientific inference and it is based on finding the simplest solution to an observed set of data (Swofford *et al*., 1996; Saitou, 1996). Maximum parsimony (MP), analysis attempts to build a tree that minimizes the number of evolutionary changes required to explain the observed data (Farris, 1983). Therefore, the optimality criterion (which in this case is minimized) is total tree length. The length of the tree is defined as the number of character state transformations (Farris, 1983; Sober, 1981). Among methods with a model of evolution, the most widely used are the methods of maximum likelihood resulting from Felsenstein's (1973, 1981) work, and Bayesian phylogenetic methods based on the Bayes equation and Monte Carlo Markov chains (MCMC) (Goloboff & Pol, 2005). In ML methods, the likelihood of observing a given set of sequence data for a specific substitution model is maximized for each topology, and the topology that gives the highest maximum likelihood is selected as the final tree (Nei & Kumar, 2000). While MP methods seek phylogenetic solutions that minimize the amount of evolutionary change required to explain a data set (anti superfluity principle, see Kluge & Grant 2006), i.e the hypothesis that have less number of evolutionary transformations. Maximum likelihood (ML) methods attempt to find solutions that have a maximum probability of being correct, given a specific evolutionary model (Swofford *et al.*, 1996). When ML is used, the logic underling is to find the model of substitution and the topology that better explain the data (usually aligned DNA sequences), for this, statistical estimation of parameters is applied (Swofford *et al.*, 1996). However, it has been claimed that it is not appropriate the use of statistical procedures to infer historical events, because these events only have occurred once and there is not sample distribution to be measured (Kluge, 1998).

Maximum parsimony (MP), is attractive because it is based on finding the simplest solution to an observed set. However, MP is criticized as being a statistically unreliable method and fails to make explicit an underlying "model" of evolution. Despite the contradictory logical foundations between statistical and non-statistical method, MP analysis is viewed as a parametric statistical method when the maximum likelihood solution is identical to the parsimony solution, it is rests on the false premise that all quantitative, numerical methods are necessarily statistical, even if only implicitly (Padial *et al.*, 2014). Another problem with the use of parsimony as an optimality criterion using molecular data, that has been broadly cited, is the Long Branch Attraction (LBA) (Felsenstein, 1978 ; Hendy & Penny, 1989; Bergsten,2005).

LBA is defined as “any situation in which similarity due to convergent or parallel changes produces an artefactual phylogenetic grouping of taxa due to an inherent bias in the estimation procedure” (Andersson & Swofford, 2004). Sequences of DNA are particularly susceptible to this distortion because there are just four possible characters, while the morphological characters in general have a broad range of variation (Bergsten, 2005. Due to the effect of LBA, the use of evolutionary models, have taken command over MP. Nevertheless, this artefact has also been reported in ML method (Kück *et al.*, 2012; Parks & Goldman 2014) and Bayesian inference (BI) method (Lartillot *et al.*, 2007). Several studies in MP analysis have successfully moderate problems related to LBA by sampling more taxa to break up and adding combining more diverse data especially morphological with molecular data and unlinked genes (Lyons-Weiler & Hoelze, 1997; Dacks *et al.*, 2002). As well as in BI, many studies have also shown how mitigated problems related to LBA by using more complex sequence evolution models and improving the model fit to the data, which reduces the model assumption violations (Lartillot & Philippe 2004; Lartillot *et al.*, 2007).

In phylogenetic analysis, the use of ML depends on three assumptions: 1) evolution is independent in different lineages and in different sites for a given tree (Felsenstein, 1981, 2004; Swofford *et al.*, 1996), and 3) the same stochastic process of substitution applies in all lineages (Felsenstein, 1981). There is a lot of criticism about this assumptions, some authors argue that the assumptions are unrealistic and/or violated in the calculus (Siddall & Kluge, 1997; Kluge, 2001; Goloboff, 2003). Users of ML analysis claimed that the method is generally robust to violations of these assumptions (Felsenstein, 1978, 1981; Goldman, 1990; Swofford *et al.*, 1996; de Queiroz & Poe, 2001). Furthermore, as ML analysis is based on a particular model of sequence evolution to infer phylogenetic relationships, interpretation of ML results have the following warning: “if the model is true, then…”. We may know which of the models best fits the data according to a model selection procedure, but how can the validity of the model itself be independently tested? Testing the validity of models, although it has been recognized as important (Goldman, 1990), is rarely done in practice (Siddall & Kluge, 1997).

The difference between ML and IB is that Bayesian approach provides probabilities for hypotheses, not probabilities of data, given a hypothesis (Lewis, 2001). Bayesian inference of phylogeny generates an approximation of the posterior probability distribution of all parameters (branch lengths, tree topology, and substitution model parameter estimates) in a phylogenetic analysis by using MCMC (Mau, 1996; Rannala & Yang, 1996). Another important difference between Ml and IB is that Bayesian approach calculation not only does imply summation over all possible combinations of branch lengths and model parameters, It also includes a prior probability density distribution of these latter variables (Huelsenbeck *et al.*, 2001), permitting that the values of parameters to be adjusted according to MCMC sampling (Brooks *et al.*, 2007). Bayesian approach requires the incorporation of a priori knowledge in terms of prior probabilities and this "prior knowledge" and this is seen as an advantage, but when prior knowledge is non-existent or ambiguous, this make it difficult to determine a particular prior distribution. The likelihood calculation used in the IB requires the same models of evolution and their associated assumptions as the ML methods described above, and thus all of the caveats about "if the model is true, then…" inherent in ML phylogeny estimation also apply to IB.

From the operational point of view, the selection of the best tree under any method (excluding methods based on distances) is not a trivial problem. The operative fact that the universe of possible trees increases dramatically with the linear increase of taxa included in a study (Cavalli-Sforza & Edwards, 1967; Felsenstein 1981), and the increasing quantity of molecular data, is a harder issue in statistical method, because of the increment in the number of parameters to be evaluated. One important issue with the use of ML is that the algorithms of the programs that implement these methods, treat the gaps as missing data, which is unrealistic and may cause false resolution in clades where parsimony simply collapses the clades because lack of support (Padial *et al*., 2014).

There are three statistical properties that have been evaluate between methods of phylogenetic reconstructions, these are consistency, efficiency and robustness (Wheeler, 2012). In terms of consistency, it has been shown that MP may be more susceptible to obtain the “wrong” tree than statistical method, when the number of changes is increased because LBA, but statistical methods are also affected by LBA. In terms of efficiency, that means the amount of data necessary for one estimator to produce one value close to the estimator, but there is not a consistently best performance of one or another method, but Steel & Penny (2000), have shown the superiority of parsimony in some particular scenarios. According to the robustness criteria, which refers to the performance of the estimators with some violations of assumptions, the conclusion is that statistical method may be superior to parsimony, when an appropriate model is selected, but in other case it would be far worse.

A short conclusion about the preference between methods of phylogenetic reconstruction is that the most important aspect should be on the consideration about of the assumptions of each method. In this study, we compare the results of Bayesian phylogenetic inference with those of MP and ML methods in the reconstruction of phylogenetic hypothesis of the *P. conspicillatus* group.

Taxon Sampling and Outgroup Selection

In order to examine the phylogenetic relationships of the *Pristimantis conspicillatus* group, we included as an ingroup, the maximum possible number of species that have been assigned to the *P. conspicillatus* group (Lynch 1994; Lynch & Duellman, 1997; Hedges *et al,*. 2008, Padial & De la Riva, 2009; Padial *et al.*, 2014) (Table 1). Samples used for whole genomic DNA extraction were obtained from multiple field trips in Colombia to many Andean locations, and we obtain tissues samples from Brazil, Peru and Venezuela in a combined effort by colleagues from Brazil (Figure 1). We generated sequence data for 98 individuals representing 34 species (Appendix 1), additional sequences were downloaded from Genbank representing in total 300 terminals in the ingroup. For outgroup taxa we used all the different species group within *Pristimantis* available in GenBank, 174 individuals representing 118 species. Moreover, we used genera placed in Brachycephaloidea: *Craugastor*, *Ceuthomantis*, *Haddadus*, *Eleutherodactylus*, Phyzelaphryne, *Adelophryne*, *Diasporus*, *Holoaden*, *Psychrophrynella*, *Bryophryne*, *Phrynopus*, *Lynchius*, *Barycholos*, *Noblella*, *Brachycephalus*, *Brachycephalus*, *Hypodactylus*, *Ischonocnema, Strabomantis* and *Yunganastes,* and finally we included *Gastrotheca cornuta* as the root. In summary, DNA sequences represent 617 terminals (Appendix 1).

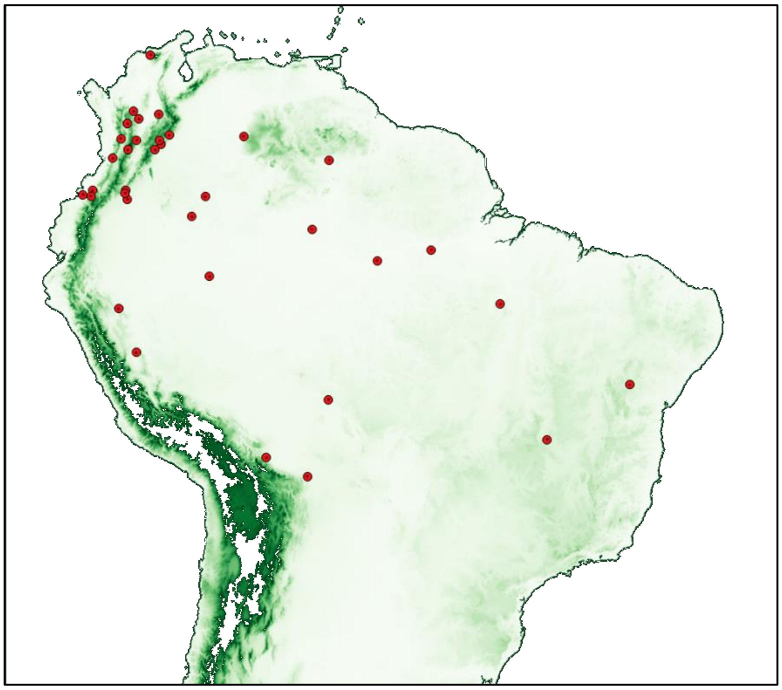

Figure 1. Red points represent the localities where specimens were collected to obtain new DNA sequences used in the present study.

Locus sampling

We targeted the complete mitochondrial ribosal RNA genes 12S and 16S along with the intervening tRNAvaline, but when this was not possible for all the organisms, we amplified one DNA fragment for 16S rRNA (16S) and 12S rRNA (12S).). We also obtained DNA sequence data from exons of two nuclear genes: portions of the nuclear tyrosinase precursor (Tyr) and recombination activating gene 1 (RAG1) and nuclear loci (RAG1, TYR) that were already partly available for Terrarana. We created a database sequence of DNA sequences deposited in GenBank, which represent loci used by previous studies to infer relationships of terrarana. Non-coding mtDNA genes include rRNA genes of the heavy strand transcription unit 1 fragment (12S, 16S and the intervening tRNAvaline, and tRNAleucine segments). Protein-coding mtDNA genes include cytochrome b (cytb), cytochrome c oxidase subunit I (COI), and NADH dehydrogenase subunit I (ND1) and subunit II (ND2), and intervening tRNAcyst. Nuclear protein-coding genes include two exons of cellular myelocytomatosis (c-myc), chemokine receptor 4 (CXCR4), histone H3 (HH3), sodium-calcium exchanger 1 (NCX1), propiomelanocortin A (POMC), recombination- activating protein 1 (RAG1), rhodopsin (Rhod), seven-in-absentia (SIA), solute carrier family 8 member 3 (SLC8A3), and tyrosinase precursor (Tyr). Non-coding nuclear genes include 28S and the intron region of the cellular myelocytomatosis gene (c-myc). (Appendix 1).

DNA extraction, amplification, and sequencing

DNA amplification was completed using combinations of primers using by Hedges *et al*. (2008); Padial *et al*. (2009) and Pinto *et al.* (2012) (Table 2). Total DNA was extracted from muscle preserved in 95% ethanol using standard phenol–chloroform extraction protocols (Sambrook *et al*., 1989). DNA amplification and sequencing methods were carried under standard protocols and are those described by Hedges *et al.* (2008). PCR was performed under the following reaction conditions: initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of 94 °C (30 seconds), 50 °C (30 seconds), 72 °C (60 seconds), and a final extension at 72 °C (7 minutes). Amplified products were sequenced by Servicio de Secuenciación y Análisis Molecular Instituto de Genética (SSIGMol_Universidad Nacional de Colombia, Bogotá) and by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea). Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software SEQUENCHER 3.0. (Gene Codes, Ann Arbor, MI, USA). Complete sequences were edited with BioEdit (Hall, 1999).

Table 2. Primers employed in this study for PCR and DNA sequencing

Primer name	**Primer sequence**	**Reference**
Sar-L	CGCCTGTTTATCAAAAACAT	Palumbi *et al.* (1991)
Sbr-H	CCGGTCTGAACTCAGATCACGT	Palumbi *et al.* (1991)
12.1L4EmodT	GAGGGTGACGGGCGGTGTGTA	
12H30EMod	CAGGAAACAGCTATGACCTACACATGCAAGTHTCMGC	
12L27	ACGTCAGGTCAARGTGCAGC	Heinicke *et al.* (2007)
16H36E	AAGCTCCAWAGGGTCTTCTCGTC	Heinicke *et al.* (2007)
16L41mod	CGCCTGTTTACCAAAAACATC	
16H37	TTACTCCGGTCTGAACTCAGATC	Heinicke *et al.* (2007)
R182	GCCATAACTGCTGGAGCATYAT	Heinicke *et al.* (2007)
R270	AGYAGATGTTGCCTGGGTCTTC	Heinicke *et al.* (2007)
Tyr1C	GGCAGAGGAWCRTGCCAAGATGT	Bossuyt & Milinkovitch (2001)
Tyr1G	TGCTGGGCRTCTCTCCARTCCCA	Bossuyt & Milinkovitch (2001)

Morphological data

We examined preserved specimens of 64 species, of these, 42 are species of the *P. conspicillatus* group, and the remaining are members of the outgroup (Appendix 5). We examined external morphology and cranial osteology. We included the external morphological characters that had been used previously to diagnose the *Pristimantis conspicillatus* group (Lynch, 1975; Lynch & Duellman, 1997; Duellman & Pramuck, 1999; Padial & De La Riva, 2009: Padial *et al., 2*009). We follow the terminology employed by Lynch & Duellman (1997).

We included cranial morphology and for this we studied cleared-and-double-stained (C&S) specimens of frogs from the herpetological collections at the Instituto de Ciencias Naturales of the Universidad Nacional de Colombia (ICN) (Appendix 2). Techniques for clearing and double-staining specimens with Alcian Blue and Alizarin Red were made following Dingerkus & Uhler (1977). We studied descriptions of cranial osteology presented by previous authors (Lynch, 1971; Lynch, 1979; Lynch, 2001; Guayasamin, 2004), and these were converted to character and character states. Other characters were discovered upon examination of the individuals. To understand and estimate intraspecific variation of osteological data, we studded eight individuals on average per species with a good number of skeletons at the ICN (see Appendix 2 and 5). To minimize effects of development, decisions about character hypothesis were taken only after examining adult females. We determined sexual condition and maturity by dissection or observation of external and internal sexual characters. The majority of characters and character states were determined by personal examination. In those cases in which the species was not available for study, the information was taken from the literature or from drawings made by JDL.

The sample of morphological data is strongly biased to: 1) diagnose and; 2) search for synapomorphy that might support the monophyly of the *P. conspicillatus* group. In this study we included the characters that have been used previously to diagnose the *P. conspicillatus* group (Lynch, 1975; Lynch & Duellman, 1997; Duellman & Pramuck, 1999; Padial & De La Riva, 2009). All the characters used by these authors are based on external morphology. Here we also evaluated the following characters: relative length of the fingers I and II, texture of skin of venter, vocal slits, dorsolateral folds, tympanic membrane, tarsal ornamentation, relative lengths of the toes III, IV and V, toe webbing, and shank length. Some character states of these morphological variants were modified.

We included cranial osteology in search of a source of evidence within the *P. conspicillatus* group, some of the characters evaluated have been used in other groups of Brachycephalids, and others are proposed herein. Beginning in the early 1980s, John D. Lynch (JDL) began a project in which he used cranial characters as a source of systematic information for frogs of the genus *Eleutherodactylus* (or as now recognized a complex of several genera). His fixation on cranial osteology led him to make drawings of skulls of many species. For some of the species included in this study it was not possible to examine cleared material, the information required was extracted from drawings by JDL.

There are also some morphological traits that have been used within *P. conspicillatus* group that we discarded. We have not taken into account color pattern (except for raphe on the throat), dorsal skin texture, finger fringes or toe fringes. Statements in diagnoses and descriptions may suggest that variation in states is discrete, however in many cases they are not, for instance, we were unable to distinguish character states proposed for dorsal coloration pattern in dorsal body pattern, patterns on posterior surfaces of the thighs, and dorsal skin textures, as all the variation observed in these traits was continuous. Duellman & Pramuk (1999) also noted that within the dorsal skin texture, the distinction between shagreen with scattered tubercles versus finely tuberculated is not discrete. The character matrix was constructed in Mesquite 3.03 (Maddison & Maddison, 2015), and analysis of character evolution were also performed in Mesquite 3.03 (Maddison & Maddison, 2015). Multistate characters were considered either as additive or nonadditive.

List of characters

1. Venter Skin.

0= Smooth; 1= with texture (granular, coarsely granular, aerolate).

Here, we refer specifically of the skin of the belly. We categorized this character as smooth if the skin of the belly has no texture. The other states apply for any type of texture on the skin of the belly: granular, coarsely granular or aerolate. The reason for this decision was that granular, coarsely granular and aerolate are not discrete.

2. Tubercle on the upper eyelid

0=absent; 1=round; 2=subconic; 3=conic.

Tubercles are modifications of the skin. In some cases tubercles in the body are not specifically named. The tubercle on the upper eyelid is located in the posterolateral quadrant of the upper eyelid (Lynch & Duellman, 1997). If the upper eyelid is simply warty or tuberculated with none of the tubercles being larger than the others, the state is defined as “tubercle eyelid absent”. Round tubercle corresponds to a shape like or approximately like a circle. Subconical and conical tubercles are tubercles that have the form of a cone. The differences between conical and subconical is that the conical tubercle is much longer than wide, and the subconical is wider than longer. Tubercle on the upper eyelid was considered additive.

3. Tubercle on heel.

0=absent; 1=round; 2=subconic; 3=conic.

The upper edge of the heel commonly bears one or more tubercles termed “heel tubercles” or “calcars” (Lynch & Duellman, 1997). Savage (1987) used the term calcar for a variety of ornamentations on the heel. Here we used that term only to refer to an enlarged tubercle as defined by Lynch & Duellman (1997). We called such structures as round tubercles if the tubercle has shape like or approximately like a circle. Sub-conic and conic tubercles are those that have the form of a cone. As for the tubercle on the upper eyelid, the differences between conical and subconical is that conical tubercle is much longer than wide. Enlarged tubercles are here considered the same as conical. No tubercles are clearly identifiable when the skin of the tarsus and the heel bears the same texture, the heel is tuberculated as is leg is considered tubercle absent. Tubercle on heel was considered additive.

4. Supra-anal warts.

0=absent; 1=present

Lynch & Duellman (1997) described the specific terms for the elevations of the skin. Warts are relative large elevations but flattened or rounded in contrast to tubercles; the base of a wart is broader than its elevation. Supra-anal warts in this study are conical warts, these are located on the top portion of the cloaca. Tubercles on upper eyelids and on heels have been used to differentiate species in *Pristimantis.*

5. Dorsolateral folds.

0=absent; 1= anterior, incomplete; 2=complete

We are following the same definition of Lynch & Duellman (1997). Dorsolateral folds are paired folds that extend posteriorly from the posterolateral corner of the orbit and lie lateral to the sacrum and the blades of the ilia. Some species have short dorsolateral folds that are not evident posterior to the sacrum and this is the case for state 1. A complete dorsolateral fold is fully developed and continuous from the posterior edge of the upper eyelid to the sacrum (Duellman & Lehr, 2009). Dorsolateral folds can be thin or strong depending on the different protuberances of the skin. We are not interested in the different textures of skin in the fold and we concentrated on whether the dorsolateral fold is present, incomplete or complete. Dorsolateral folds was considered nonadditive.

6. Fold on inner edge of tarsus.

0=absent; 1=weak; 2=strong; 3= flaplike

A tarsal fold is a fold extending from the distal portion of the tarsus segment of the hind limbs for one half to the three quarters of its length (Savage, 1975). We made a slight modification on the definition of this character; we considered state 1, if a low, narrow rounded or elongate ridge or tubercle is observed. Strong; a well-developed narrow tarsal fold, having considerable vertical dimension. Flaplike; extremely well developed narrow tarsal fold with both margins free and the entire structure flaplike and movable. Duellman (1963) and Stuart (1963) suggested that tarsal features could be used to separate the Atlantic populations of *Craugastor rhodopis*. Lynch (1969) examined the ornamentation of tarsal folds in 361 specimens of *C. rhodopis*, yielding eight different classes of tarsal ornamentation, ornamentation with prominent tubercles followed by a strong fold with a prominent round tubercle in the middle of the fold have not been observed in any species of the *P. conspicillatus* group and we have not observed these conditions in any species of *Pristimantis* distributed in Colombia. The tarsal folds are absent when no ornamentation is found in the interior edge of the tarsus and when the surfaces of the tarsus are completely smooth. Fold on inner edge of tarsus was considered additive.

7. Tympanum size

0=tympanum size reaches and surpasses the lower eyelid (tympanum of moderate size); 1= tympanum size passes the lower eyelid and reaches the labial commissure (large tympanum). (Figure 2). Lynch & Duellman (1997) discussed the variety of "tympanum" or "external ear" conditions and empathized that these are a combination of structures. Here, we are only interested in the length of the tympanum using the lower eyelid as a point of reference.

8. Vocal slits in males

0=absent; 1=present

This is a secondary sexual character and the determination of adulthood makes difficult the observation. We did not take into account the differences in length between the middle lateral base of the tongue and the angle of the jaws (Duellman & Lehr, 2009). We are only interested in the absence or presence of the vocal slits. Males of *Pristimantis* species have other two secondary sexually characters: vocal sac (vocal apparatus) and nuptial pads. We did not evaluate these features in this study. Nuptial pads are evident depending on the hormonal status of the male. Although we did not evaluate nuptial pads within the *Pristimantis conspicillatus* group, males of the species *P. dundeeii*, *P. fenestratus*, and *P. samapaitae* have double nuptial pads on thumbs.

9. Relative length of the inner fingers

0=First finger longer than second; 1=First finger shorter than second

The correct way to evaluate the relative lengths of fingers I and II has been assessed by addressing these two fingers together. As a Grant *et al.* (2006) noted, mistakes can be made in describing the relative lengths of these fingers when bringing finger I further toward finger II (or vice versa). Explanations about why a finger is shorter than the other were not addressed in this trait.

10. Basal webbing

0=absent; 1=basal; 2=moderate webbing; 3=extensive webbing

Webbing is measured in reference to subarticular tubercles in the manner proposed by Savage & Heyer (1967) and as modified by Myers & Duellman (1982). Lynch & Myers (1983) differentiated four types of webbing: webbing absent or basal, moderate webbing, extensive webbing and very extensive webbing. These kinds of webbings were defined on the basis of the relative position of the subarticular tubercles of toes III, IV and V mainly.

We made a modification on the definitions of Lynch & Myers (1983), the basal webbing is defined based on the relative position of the subarticular tubercles of toes III and IV. Additionally, we separated webbing absent or basal as two different states. No webbing between the toes is defined as absent, if the webbing is present and encompasses the basal tubercle on toes III and IV the state is defined as basal webbing. Moderate webbing occurs when the webbing reaches or encompasses the two subarticular tubercles of toe III and the basal tubercle of toe IV. Extensive webbing is present when webbing reaches all the subarticular tubercles of toe III and IV except for the distal subarticular tubercle of toe IV. Very extensive webbing occurs when webbing reaches all the subarticular tubercles of toes III and IV with only the penultimate phalanx of toe IV being free of web (
Figure 3).

At the moment there are no studies on the variation on the length of the webbing in terrarana species. It is unknown whether this involves one or more characters. We decided to define the character states based on the extension of the webbing between the toes III and IV. Webbing was considered additive.

11. Ungual flap in the dorsal surface of toes III and IV

0=not indented; 1=indented

A distinctive pair of dermal scutes on the dorsal surfaces of the digits was a character used by Noble (1926) to link all the dendrobatids. Lynch (1976) mentioned the presence of scutes or indented ungual flaps in some groups of the genus *Eleutherodactylus* in southeastern Brazil but not in the *Eleutherodactylus ramagii* group. Lynch (1979) differentiated the digits of the dendrobatids from those of the *Eleutherodactylus* by having flats (scutes) undercut (Figure 4). Hedges *et al.* (2008) placed the *E. ramagii* group in the *Ischonecnema ramagii species* series. Currently, these species fall within the *Pristimantis conspicillatus* group (Canedo & Haddad, 2012). We are aware that this flap is a structure that can help differentiating species of the genus *Ischonecnema* from other Brachycephalid (even *Dischidodactylus*). Dermal modifications (dics, flaps, papillate) in Brachycephalids are generally most strongly expressed in the level post_axial to smaller extent on the pre_axial, for this reason, we proposed the presence of an indented ungual flap at least on toes III and IV.

Relative lengths of toes III and V

Lynch & Duellman (1997) pointed out that the species group of the now dismantled genus *Eleutherodactylus* can be assembled into three Conditions (A, B and C), based on variations in the lengths of the third and fifth toes. Toe V shorter than toe III was denominated Condition A, when toe V is much longer than toe III, Condition B occurs when toe V is longer than toe III, and when the disc on toe V extends to the level of distal the subarticular tubercle on toe IV was called Condition C (Figure 5). This Condition B is variable in that in some species toe V reaches about to the level of the penultimate subarticular tubercle on toe IV and in others toe V reaches about to the half way between the distal and penultimate subarticular tubercle on toe IV.

Arroyo *et al* (In prep) evaluated the variation in lengths of toes III, IV and V in some species of the terrarana in order to search for characters that can be used as evidence of phylogenetic relationships within the genus *Pristimantis.* According to these results, the character proposed by Lynch & Duellman (1997) is discarded and new characters are proposed. The new proposal is based on the evidence that in some species of Brachycephalids the length of the proximal phalanges on toe IV is reduced and the length of distal phalanges on toe V is increased.

12. **Toe V reaches about or more than half way between the distal and penultimate subarticular tubercle on toe IV** (Figure 6a).

0= toe III does not extend beyond to the distal level of penultimate subarticular tubercle on toe IV; 1= toe III reaches and passes penultimate subarticular tubercle on toe IV.

13. **Toe V does not extend beyond the penultimate subarticular tubercle on toe IV** (Figure 6b).

0= toe III is longer than toe V; 1= toe III is smaller than toe V

Hypothesis of character 12 y 13 were considered additive.

14. **Shank size**

0=small (< 46mm); 1=medium (between 48 and 59mm); 2=large (>60mm)

Pristimantis conspicillatus species group have been recognized for have long legs (Lynch, 1975; Lynch & Myers, 1983). We took all the means of the proportion HW/SVL and plotted them in order to search for discontinuous value to differentiate long and short legs (Figure 7). Shank size was considered additive.

15. Gular stripe

0=absent; 1=present

The throat is dusky gray to dark brown with white flecks and a narrow or broad white stripe. This color pattern on the throat is distinctive in *Pristimantis epacrus, P. fallax, P. gutturalis, P. lanthanites* and *Craugastor fitzingeri*.

16. Directionality of the alary processes

0=dorsally; 1=posterodorsal

The terminus of the alary process of each premaxilla has the shape of an inverted V. The alary process is directed anterodorsally, dorsally or posterodorsally (Lynch, 1971). We only categorized dorsally and posterodorsally because the anterodorsal condition in *Pristimantis* has not been observed (Figure 8). This directionality of the alary processes was identified (and drawn) by Lynch (1989) in one individual of *Oreobates discoidalis*.

17. Separation between the nasal bones.

0= the nasals are separated, without any contact; 1= the nasals are separated medially but half or more of their lengths is in contact. (Figure 9)

18. Contact between the nasals and the frontoparietals (sphenethmoid visible or not visible dorsally).

0=nasals and frontoparietals not in contact, sphenethmoid visible dorsally; 1= nasals and frontoparietals in contact, sphenethmoid not visible dorsally.

The nasals are in broad contact with the anterior edge of the frontoparietals, and when these bones are in contact, the sphenethmoid is not visible dorsally. In some cases, the nasals and the anterior edge of the frontoparietals are in broad contact but the sphenethmoid is slightly visible, this is because there is a posteromedial separation of the nasals. (Figure 10)

19. Frontoparietals fused to the prootics.

0=not fused; 1=fused.

Frontopariets fused to the prootics is evident when no suture between the frontoparietal and prootic. There is some reasons to be cautious with this character; this character can be misinterpreted as frontoparietal-exoccipital fusion (Lynch, 2001). The data set for frontoparietal-prootic fusion consist of Lynch (1971) reporting in 32 West Indian species and nine Mexican species, Flores (1994) reporting in 21 Antillean species and Joglar (1989) reporting in 56 Antillean species. Lynch (2001) reviewed the fusion between the frontoparietal and the prootic and re-examined some specimens studied by Joglar (1989) and found a suture between the frontoparietal and exoccipital in some species and concluded that there was no a frontoparietal fusion and reduced the taxa with fusion, in which he recognized such fusion in the following terraranas species of the north of the Andes: *Pristimantis colodactylus, P. bicumulus*, *P. danae*, *P. gracilis*, *P. nicefori*, *P. platydactylus*, *P. simonboliviari*, *P. vicarious*, *P. vidua* and *Tachiromantis douglasi*, and in 13 spp. of Middle America and 64 Antillean species now allocated to the genus *Eleutherodacyulus*.

20. Cranial crests.

0=absent; 1=present.

Trueb (1970) observed that the posterior margin of frontoparietals ended in an upturned flange termed the occipital crest. Within Brachycephalidae cranial crests have been recognized by Lynch (1975, 1981, 1986, 1992, 1995) as a upturned lateral margins of the frontoparietals. This structure has been classified as: prominent, massive or low. Cranial crests also have a variant form, whether the crests take their origin on the lateral edges or whether they are inset. Here we are focused only in the absence or presence of cranial crests disregarding the importance of the variation within them, such as width, height and position. Lynch (1995) suggested that smoother cranial crests may represent the lost of bony ornamentation or ontogenetic changes. The cranial crests are better developed in adults than in juveniles.

21. **Anteromedial exposition of the frontoparietal.**

0=not exposed; 1=exposed

The paired frontoparietals do not articulate along the midline. They diverge antero and posteromedially from one another, exposing the frontoparietal fontanelle anteromedially.

22. **Ornamentation along the dorsal edge of the zygomatic and the otic rami of the squamosal bones (short ridges, bony tubercles).**

0=absent; 1=present

An ornamentation is defined as the presence of serrations along the dorsal edge of the squamosal. (Figure 10)

23. **Length of the zygomatic and otic rami of the squamosal** in lateral view

0= otic ramus longer than zygomatic; 1= otic ramus shorter than zygomatic ramus; 2= equal. (Figure 10).

The anterior ramus has more variation (Figure 10). Length of the zygomatic and otic rami of the squamosal was considered nonadditive.

24. Shape of the squamosal's ventral ramus.

0= Posterior edge of the ventral ramus of squamosal curved; 1= Posterior edge of the ventral ramus of squamosal not curved

Inspecting the drawings of skulls made by JDL, a detail caught the attention when the ventral ramus of the squamosal is curved. Under, stereo we noted that the curve is more prominent in the posterior edge of the ventral ramus of the squamosal.

25. Lengths of the otic rami of the squamosal

0= the otic ramus of the squamosal does not extend beyond the crista parotica dorsally; 1=the otic ramus extends beyond and invests the crista parotica dorsally.

In most of the species, the otic ramus of the squamosal is longer than the zygomatic ramus. We are interested in knowing how much is the extension posteromedially, therefore we used the crista parotica as a point of reference. In all the species of *Pristimantis conspicillatus* species group studied, the otic ramus of the squamosal does not extend beyond the crista parotica dorsally. The otic ramus extending beyond and investing the crista parotica dorsally is observed in the genus *Craugastor*, *Strabomantis* and in the *P. devillei* and *P. myersi* groups (Figure 10).

26. Shape of the dentigerous process of the vomer

0= triangular, with one side bearing an almost 90 degrees angle; 2= triangular, lacking any side of 90 degrees angle.

All the species sampled have vomers. Each vomer has pre and postchoanal process and bears a dentigerous proceses. The dentigerous processes lay posteromedial to the choanal opening, and are triangular in outline. We observed that the species shared dentigerous processes that are triangular in outline, however the "triangle" can vary in shape, and this depends on the angles of "triangle". All the species of the *P. conspicillatus* group studied have triangular dentigerous process of the vomer, with one side bearing an almost 90 degrees angle. The state (2) is observed in different species of the genus *Pristimantis* (*P. curtipes*, *P. thymelensis*, *P. galdi*, *P. orestes*, *P. unistrigatus*, *P. nervicus*). (Figure 11).

27. Relative length of the dentigerous process of the vomer

0= the dentigerous process does not reach any level of the palatines; 1= the dentigerous process reaches the palatines; 2= the dentigerous process overlaps and surpasses the entire structure of the palatines (the dentigerous process are visible in dorsal view). The *Pristimantis conspicillatus* species group has a prominent dentigerous process that is triangular and extends posteromedially. Teeth are evident on each dentigerous process, and they are at least eight. In this study, we quantify the extent of dentigerous process, relating the position of the dentigerous process relative to the palatines (Figure 11 and Figure12). Relative length of the dentigerous process of the vomer was considered nonadditive.

28. Size of the palatines

0= the distance between the palatines is equal or greater than half the length of one of the palatines, 1= the distance between the palatines is less than half the length of one of the palatines. The paired palatines invest the ventral surfaces of the planum antorbitale at the anteroventral margin of the orbit (Guayasamin, 2004). Here we are interested in the size of the palatines, whose length can be expressed in how much of the surface of the sphenethmoid is invested by the palatines, in the ventrolateral surface of the sphenethmoid (see Guayasamin, 2004). We considered the palatines short when the distance between them is equal or greater than half of the length of one of the palatines. The size of the distance between them reflects how extensive the palatines (Figure 12) are.

29. Length of the sphenethmoid (in ventral view).

0= sphenethmoid does not reach the postchoanal process; 1= sphenethmoid reaches and passes the postchoanal process but does not extend to a point anterior to the nasals; 2= sphenethmoid reaches and passes the anterior edge of the nasals. We decided to measure the variability of the size of the sphenethmoid in ventral view because in dorsal view the frontoparietals are not a good indicator to properly quantify the length of the sphenethmoid, therefore we used the relative position of the vomers as a point of reference, specifically the postchoanal process. Length of the sphenethmoid was considered nonadditive.

30. The cultriform process length

0= the cultriform process does not reach the palatines; 1= the cultriform process reaches the palatines; 2= the cultriform process passes the palatines and reaches the postchoanal process (Figure 12). The cultriform process length was considered additive.

31. Length of the anterior ramus of the pterygoids

0= anterior ramus of the pterygoids does not reach the anterior edge of frontoparietals;

1=anterior ramus reaching and passing the anterior edge of frontoparietals

The paired pterygoids are edentate and triradiate (with anterior, medial, and posterior rami), these articulate laterally at two points on the maxillary arch and medially they may (or may not) rest on the otic capsule. The anterior ramus extends anterolaterally and it is always longer than the medial and posterior rami (Lynch, 1971). The contact between the anterior ramus of the pterygoids has been used as a taxonomic character (see Tihen, 1962 and Lynch, 1971). We observed a long anterior ramus of the pterygoids but it do no reach the palatines, and we considered the anterior ramus of pterygoids as "long" when (in dorsal view) they reach and extend beyond the anterior margin of the frontoparietals. In ventral view, the distance between the anterior ramus and the palatines are minimal although there is not overlap between them (Figure 13).

32. Fusion of the sacrum and the eighth presacral vertebrae

0=not fused; 1=fused

The vertebral column consists of eight presacral vertebrae and the sacrum (not fused). The sacrum and vertebrae VIII are fused with no traces of sutures in zygapophyseal or central regions (fusion present).

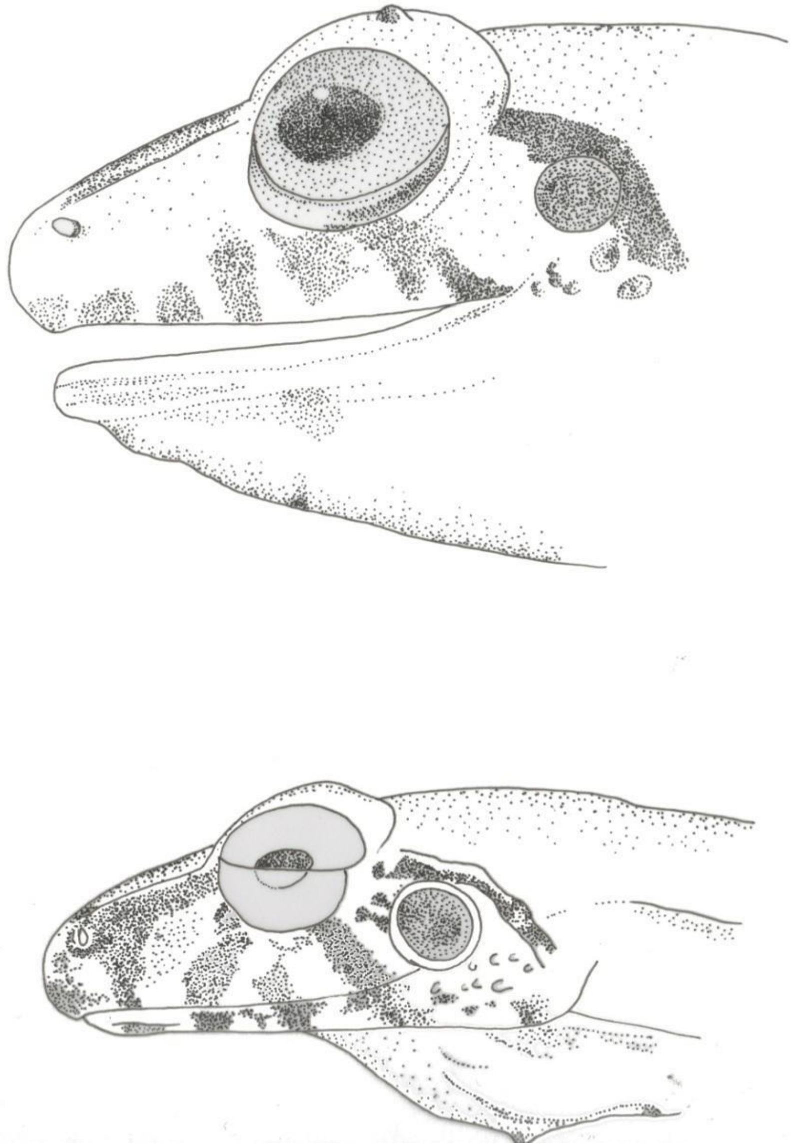

Figure 2. Lateral view of heads showing comparative size of tympanum taking the lower eyelid as a point of reference. Top: *Pristimantis savagei*,

N21383, with a large tympanum that passes the upper eyelid but not reaches the labial commissure. Bottom: *Pristimantis fenestratus*

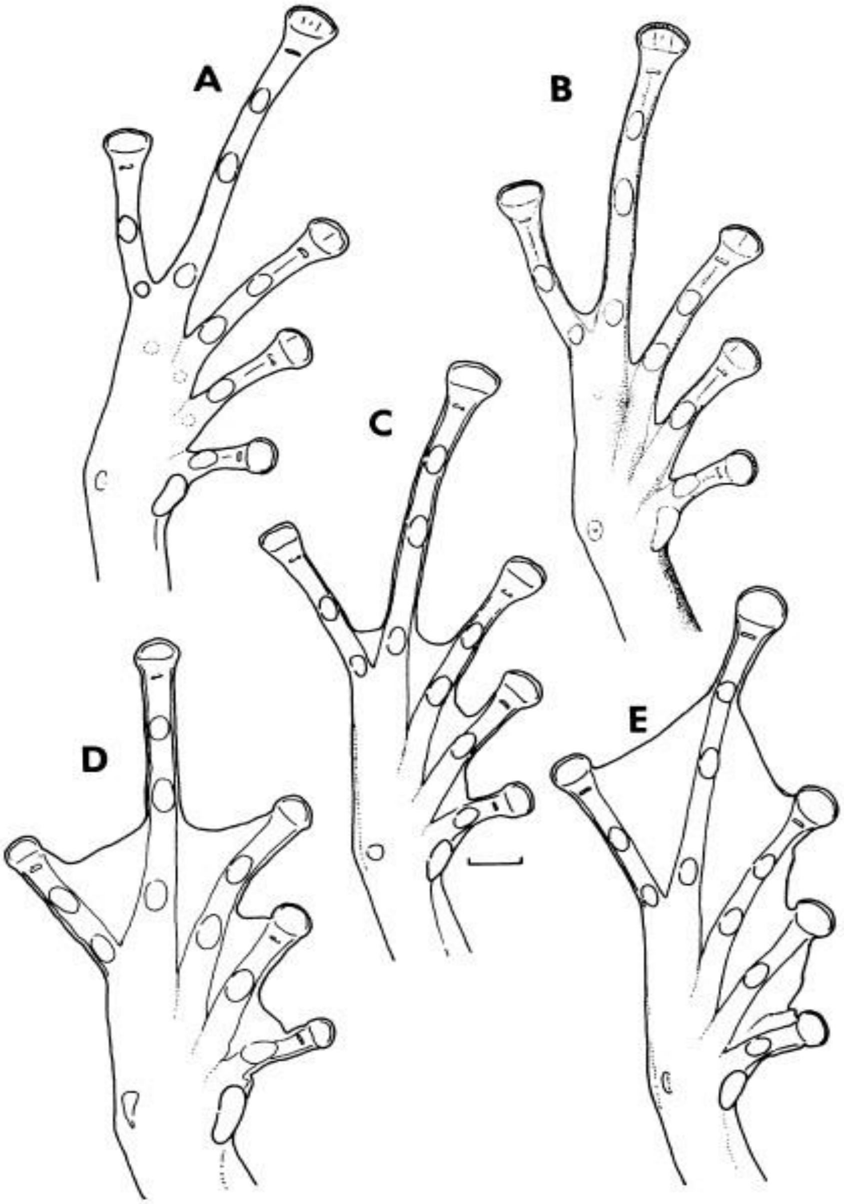

Figure 3. Plantar view showing variation in toe webbing. A. No webbing. B. Basal webbing. C. Moderate webbing. D. Extensive webbing. Figure taken from Lynch & Myers (1983).

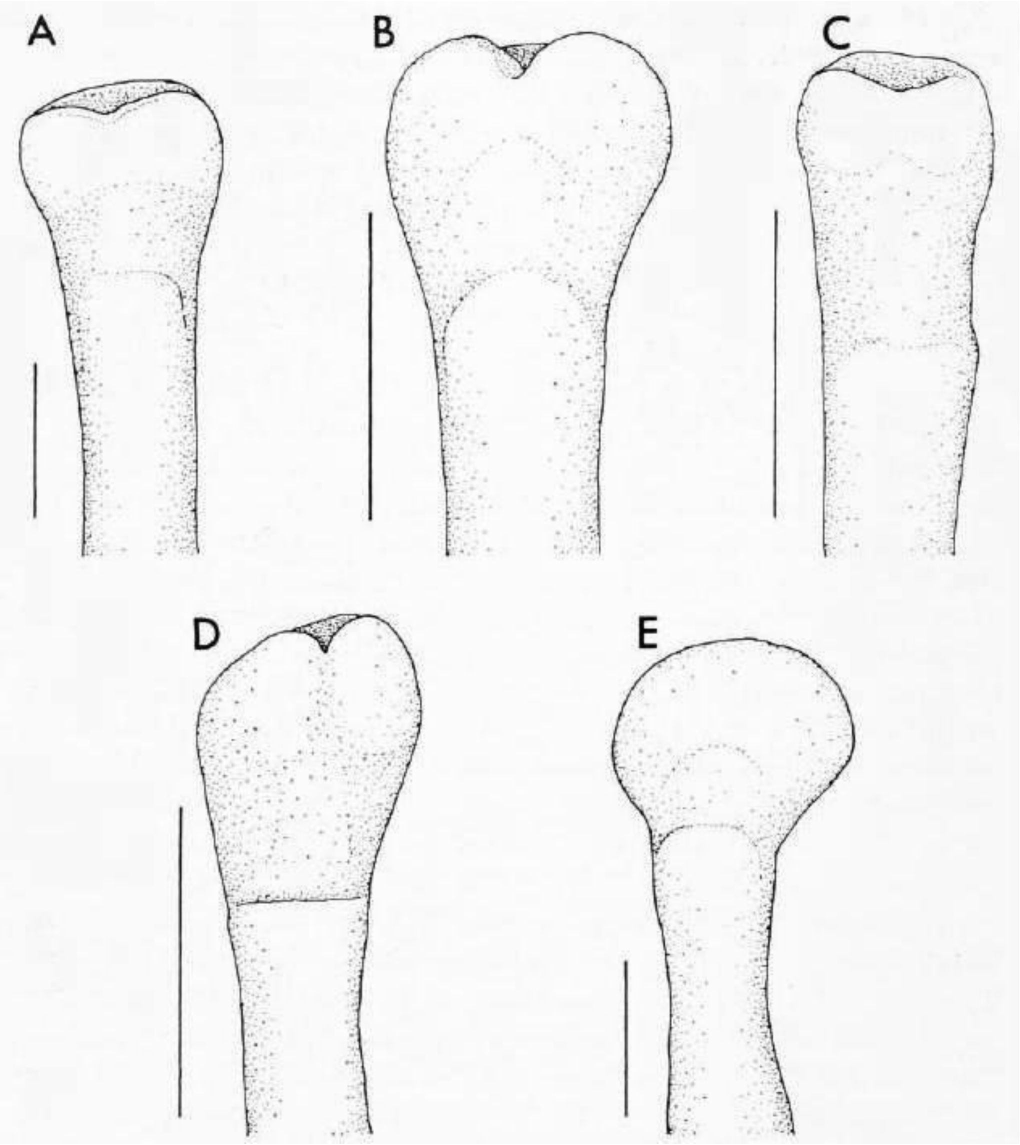

Figure 4. Dorsal view of digital pads of A) *Ischnocnema guentheri,* 3rd finger right hand KU 92816, B) *I. nigroventris* 4th toe right foot KU 92734, C) *I. octavioi* 4th toe left foot KU 92827, D) *I. parvus* 4th toe left foot KU 92831, E) *P. fenestratus* 3rd finger left hand WACB 9814. Scale equals 1 mm. Figure modified from Lynch (1976).

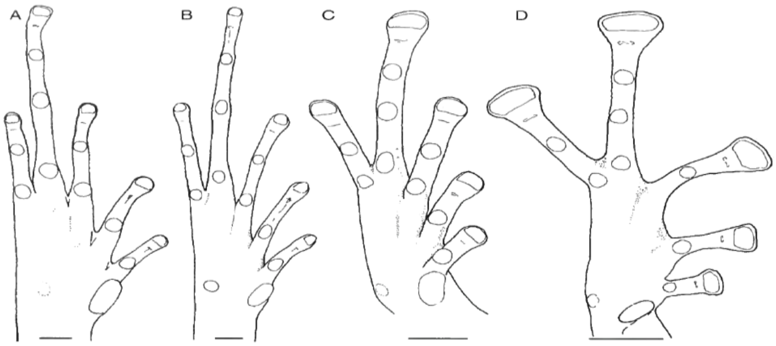

Figure 5. Plantar views of feet showing variation in lengths of toes III and V. A) *P. cerastes*, UVC 8429 Toe V shorter than toe III (condition A), B) *P. babax* USNM 285970 Toe V slightly longer than toe III (condition B), C) *P. duellmani* USNM 286044 Toe V longer than toe III (also condition B), D) *P. ornatissimus* KU 119745 Toe V much longer than toe III (condition C). Figure taken from Lynch & Duellman (1997).

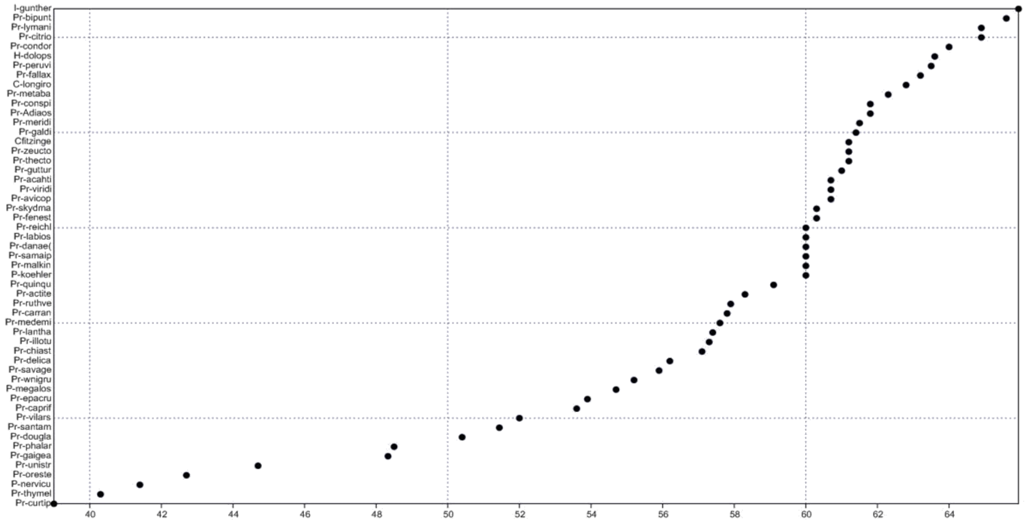

Figure 6. Shank size large >60 mm.

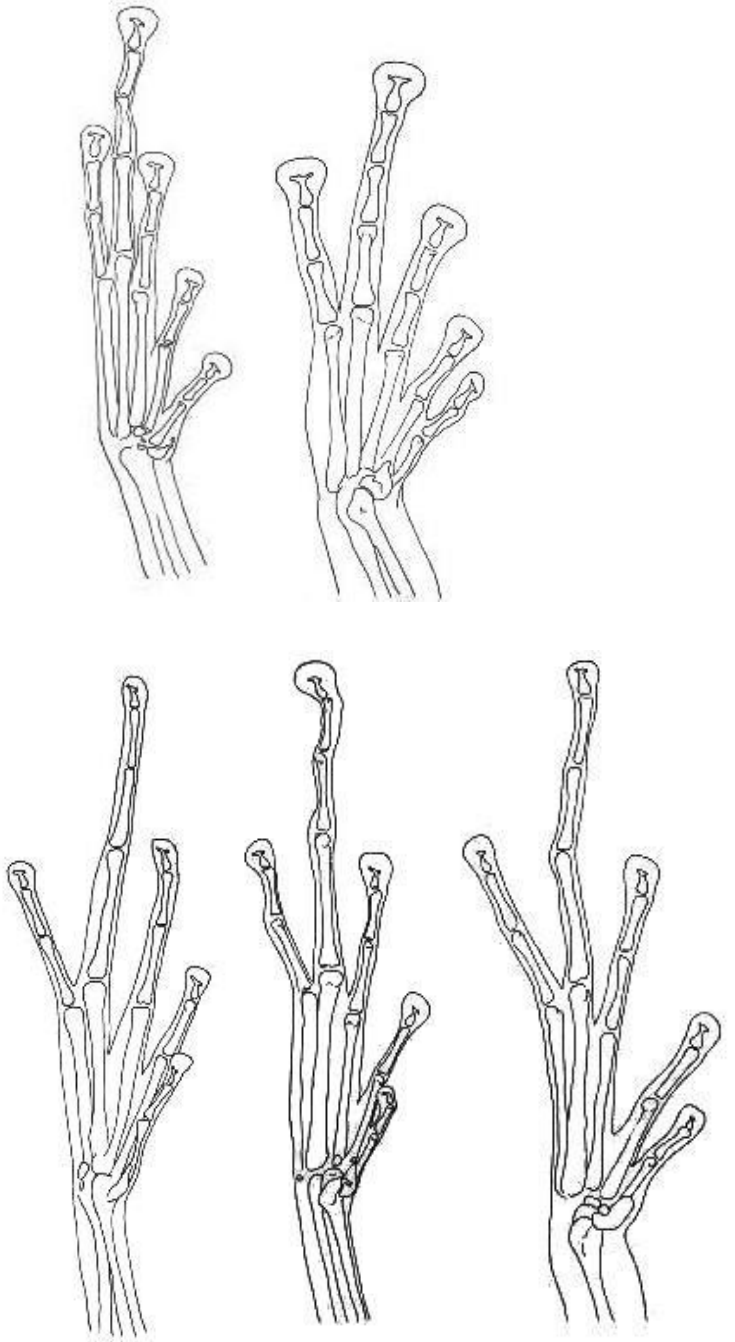

Figure 7. **Upper**. Character 12. Toe V reaches about or more than half way between the distal and penultimate subarticular tubercle on toe IV. 0= toe III does not extend beyond to the distal level of penultimate subarticular tubercle on toe IV (left); 1= toe III reaches and passes penultimate subarticular tubercle on toe IV (right). **Bottom**. Character 13. Toe V does not extend beyond the penultimate subarticular tubercle

on toe IV. 0= toe III is longer than toe V (left); 1= toe III is smaller than toe V (middle and right)

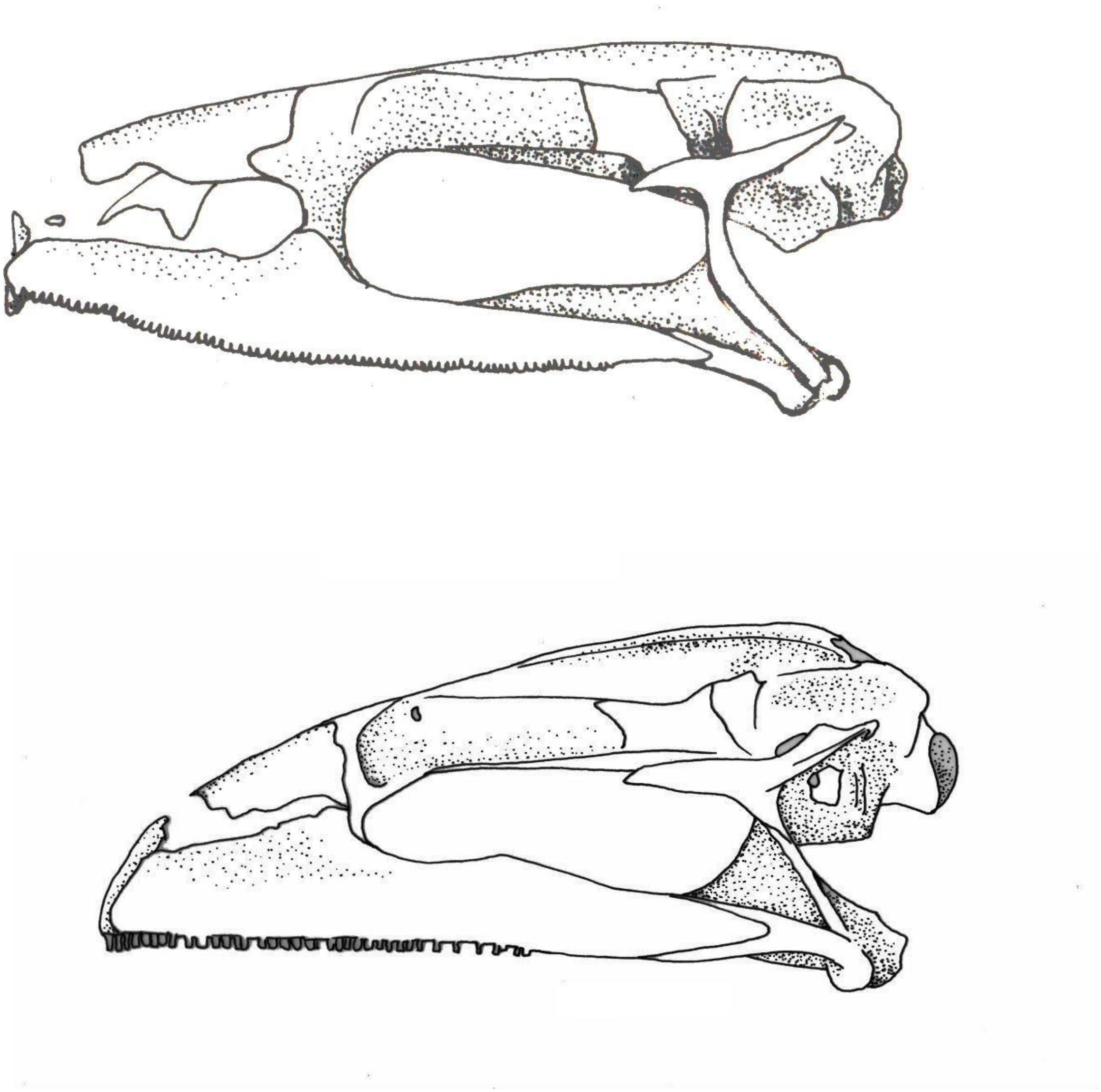

Figure 8. Lateral views of the skulls. Top: *Pristimantis vilarsi*, ICN 11907; Bottom: P. insignitus, ICN 13164. Illustrating the directionality of the alary processes and size of the zygomatic and otic rami of the squamosal.

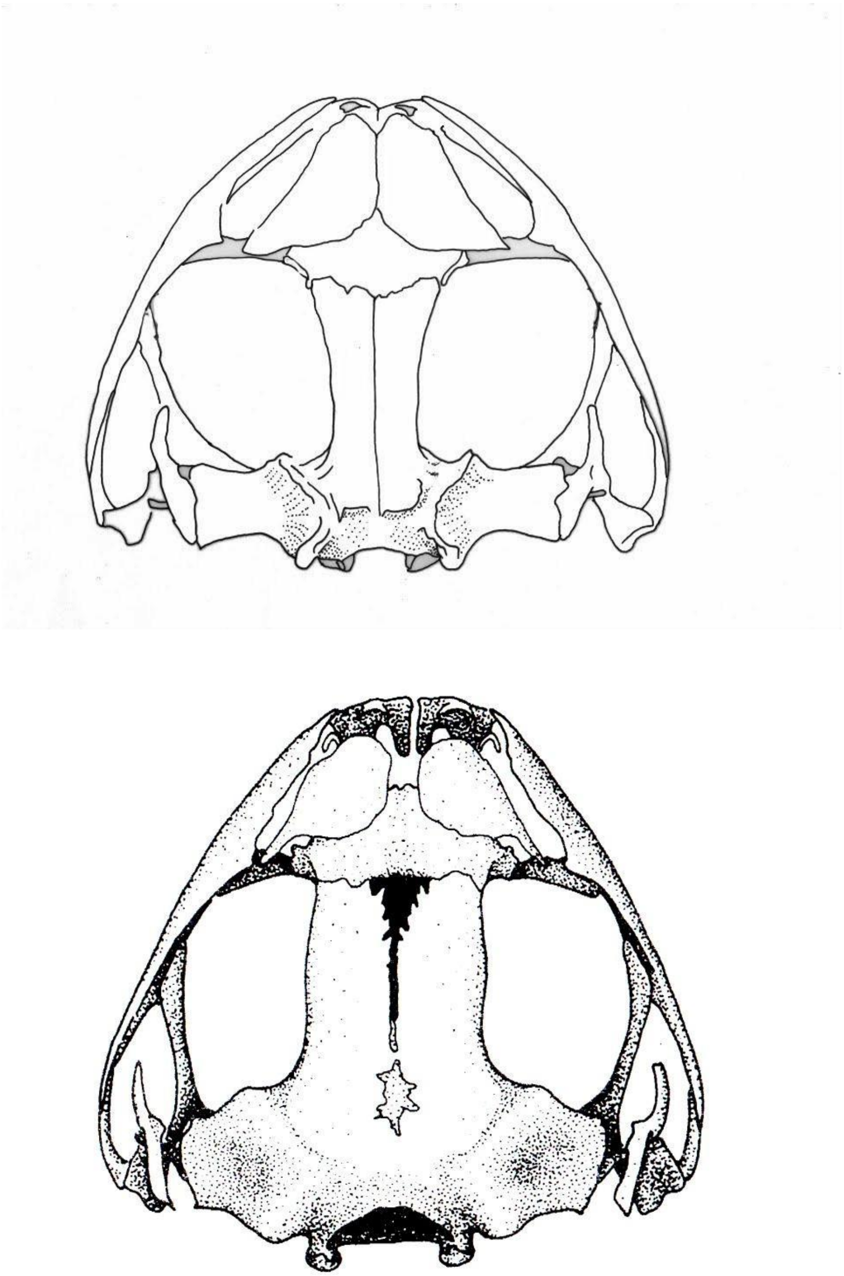

Figure 9. Dorsal view of skulls. Separation between the nasals bones. Top: *Pristimantis lymani*, KU 11905; Bottom: *Pristimantis orestes*, KU 15102. Figure taken from Lynch (1979).

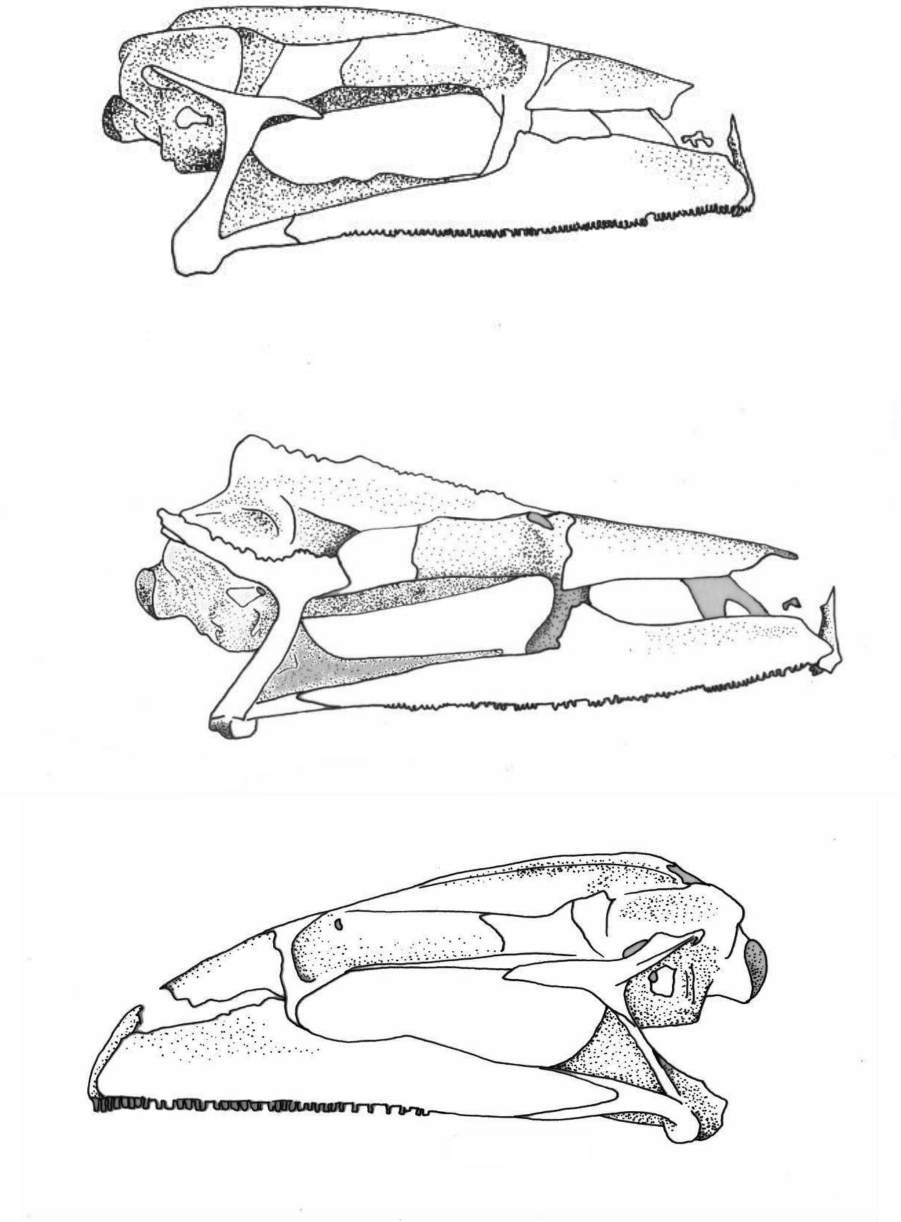

Figure 10. Lateral views of skulls. Ornamentation along the dorsal edge of the squamosal, and length of the zygomatic and otic rami of the squamosal. Top: *Pristimantis actites*, KU 288913; middle: *Pristimantis galdi*, USNM 212258, showing otic ramus extends beyond and invests the crista parotica posterodorsally; bottom: *Pristimantis insignitus*, ICN 13164, Illustrating otic ramus shorter than zygomatic.

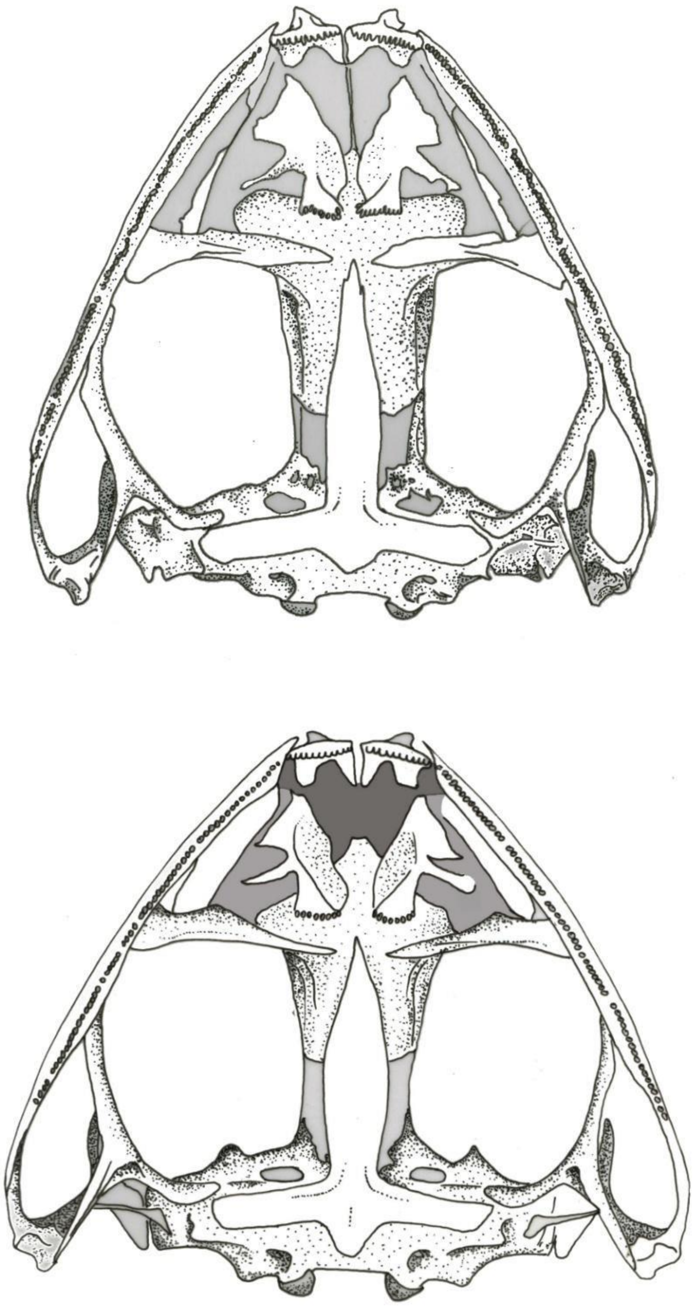

Figure 11. Top: *Pristimantis lanthanites*, KU 15307, the dentigerous process does not reach any level of the palatines. Bottom: *Pristimantis achatinus* 11306, the dentigerous process reaches the palatines.

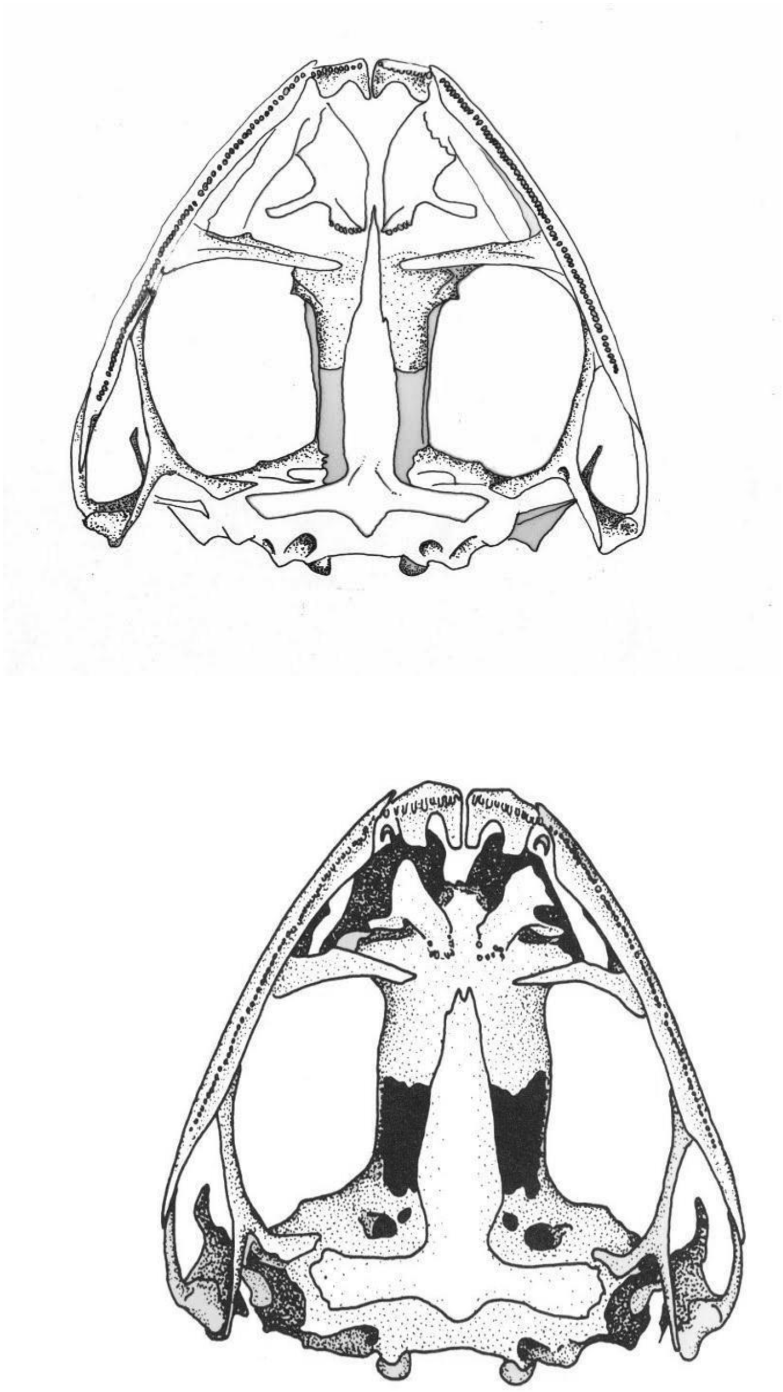

Figure 12. Illustrating the size of palatines and the cultriform process length.

Top: Pristimantis medemi, *ICN 14588; Bottom;* P. orestes, *figure taken of Lynch (1979).*

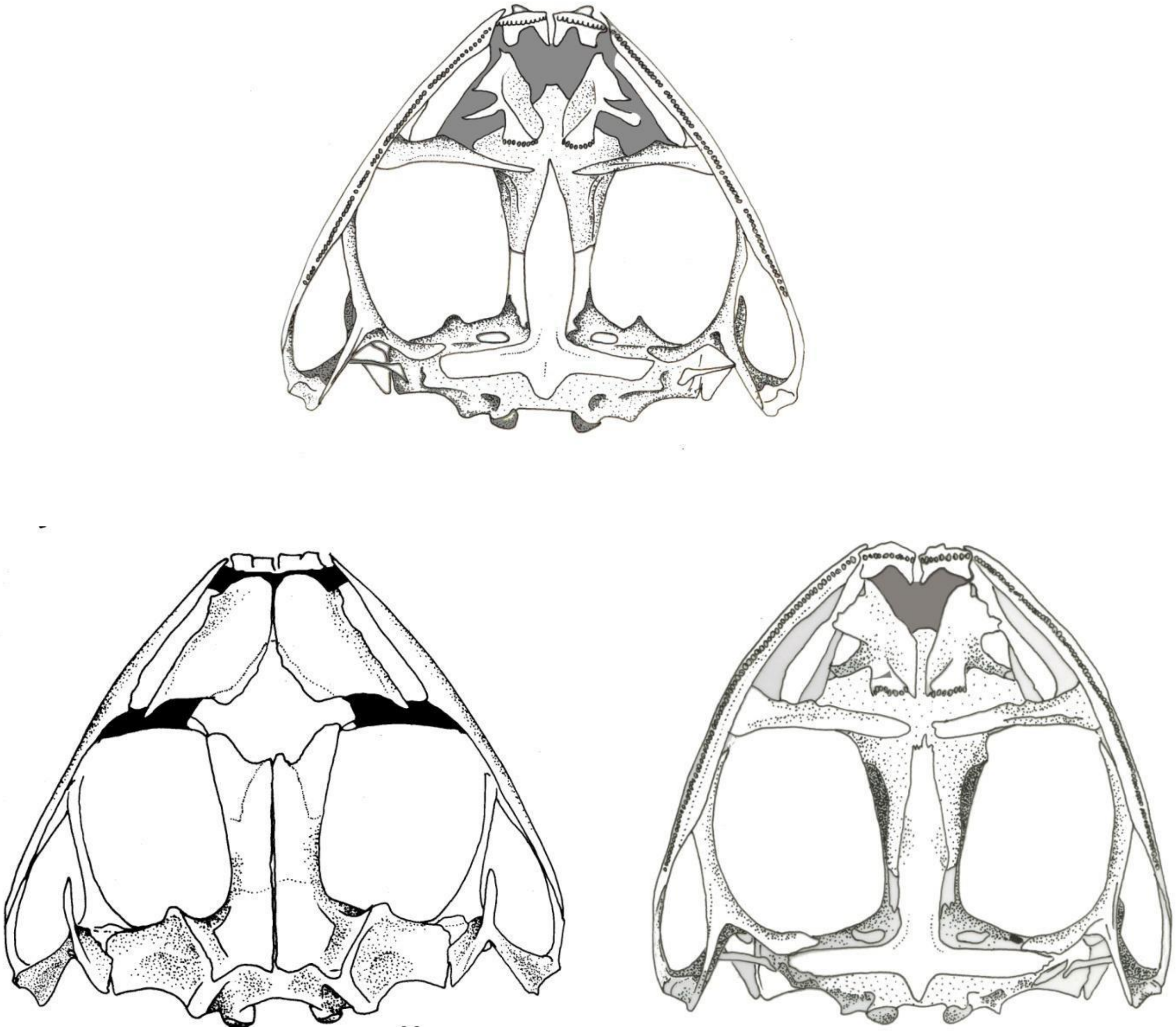

Figure 13. Length of the anterior ramus of the pterygoids. In ventral view, the distance between the anterior ramus and the palatines are minimal although there is not overlap between them. *Pristimantis w-nigrum* KU 17094 (bottom left). *Pristimantis actites* JDL 10760.

Phylogenetic analysis

We performed four different analysis: 1) MP (molecular data), MP (molecular + morphological data), ML and IB. We aligned the sequence matrix through MAFFT v6 on line (Katoh *et al.*, 2005), which is considered appropriate for alignments that consist of large numbers of sequences (Katoh *et al.*, 2005). For the mitochondrial regions we employed the alignments generated with L-INS-i strategy and for nuclear regions we employed the alignments generated with FFT-INS-i strategy. We obtained a final 17635 bp alignment.

Maximum parsimony. For the two MP analysis, we weighted all transformations equally and treated gaps as a missing data. Tree search were performed in the software TNT (Goloboff *et al.*, 2008). Searches were done using sectorial searches, tree drift and tree fusing (Goloboff, 1999), including driven searches until a stable strict consensus was reached at least 15 times. Bootstrap analysis was estimated with 1000 repliques (Felsenstein, 1985). We considered relationships strongly supported when MP bootstrap percentages equalled or exceeded 70% (Hillis & Bull, 1993).

Maximum likelihood. The same MAFFT alignment used for the parsimony analyses was used in the maximum likelihood analyses. Maximum likelihood analyses (maximum average likelihood in the sense of Barry & Hartigan, 1987) using the optimal partition scheme and substitution model were performed in GARLI 2.0 (Zwickl, 2006). Because we have a data matrix similar to that of Padial *et al.* (2009): 18 genes in a terrarana data matrix, we selected the same optimal partition scheme that they found in their analysis, where PartitionFinder identified the 2-partition (mtDNA/nuDNA) scheme with the GTR + G substitution model for both partitions to be the optimal model. Bootstrap analysis was estimated with 500 replicates.

We used two criteria to infer the existence of distinct species: (1) cladogram topology (cladistic distance), and (2) uncorrected pairwise distance of parcial 16S sequences within and between localities and/or closely related species. Genetic distances (p distance) were also computed using MEGA 6 (Tamura *et al.*, 2013) and are shown In Appendix 3.

Bayesian analyses. We performed Bayesian phylogenetic analyses using BEAST 1.8.3 through CIPRES portal. Again, the model GTR + G substitution model was used for both partitions. We run an analysis for 70 million generations, sampling trees and parameter values every 1000 generations. Convergence and stationarity of the Markov process were evaluated using Tracer 1.3 (Rambaut & Drummond, 2004). The first 1 million generations were discarded as burn-in.

Divergence times. Divergence times along with phylogenetic relationships were estimated for the complete data set using a Lognormal relaxed Bayesian clock implemented in BEAST 1.8.3 (Drummond & Rambaut, 2007). As calibrations constraints we used the stem age of *Pristimantis* and Gastrotheca sensu Heinicke *et al.* (2007), 24.45 (C.I. = 17.30–34.82) Million years ago (Ma) and 56.79 (C.I. = 43.52–78.13) Ma respectively.

Results

Parsimony analysis with molecular data

Driven searches in TNT found 38 optimal trees of 76253 steps (Figures 14, 15 and 16). The *Pristimantis conspicillatus* group was recovered as a monophyletic although with low support.

The hypothesis of a monophyletic *P. conspicillatus* group includes the following twenty three species: *P. achatinus*, *P. bipunctatus, P. buccinator, P. citriogaster, P. chiastonotus, P. condor, P. conspicillatus, P. koehleri, P. fenestratus, P. gaigei, P. gutturalis, P. lymani, P. malkini, P. paulodutrai, P. peruvianus, P. ramagii, P. samaipatae, P. skydmainos, P. terraeboliviaris, P, ventrigranulosus, P. vilarsi, P. vinhai,* and *P. zeuctotylus.*

The *Pristimantis conspicillatus* groups is divided into three main clades, each of these three clades received low support (Figure 16). The clade A is sister of all other species of *P. conspicillatus* group and is composed of *P. gutturalis* and *P. terraeboliviaris*, and a clade of unnamed species. *Pristimantis gutturalis and P. terraeboliviaris* are sister species and these two are sister to a clade containing at least five undescribed species of the Atlantic Forest. *Pristimantis gutturalis* has been registered from the state of Amapá, southern French Guyana and eastern Surinam, therefore we consider the sample (*P. gutturalis* 577PG) from Mitaraka (French Guyana), close to the distributional range of *P. gutturalis*. The specimens identified as *P. gutturalis* (MT46 and MT108) from Pará and Amazonas (Brazil) are misidentified, we are going to refer as a "*P. gutturalis*". The voucher used for *P. terraeboliviaris* is from Tobago, where *P. terraeboliviaris* has been confused with *P. charlottevillensis* (Murphy, 1997). We have some doubts about the identity of the specimen used below under the name *P. terraeboliviaris*.

Pristimantis lymani from Loja (Ecuador) is sister to *P. achatinus* from the Pacific coast of Colombia and Ecuador. The topology shows existence at least of two species-level lineages within nominal *P. achatinus.* Populations *of P. achatinus* from the Pacific coast of Colombia and Panama (Darien), and from moderate highlands of Cordilleras Occidental and Central of Colombia are two different lineages, this last one we are going to refer as a *P. aff. achatinus*. Furthermore, the *P. achatinus* from the Colombian Choco might be a complex of more than one species. We have found differences at the level of the skull: cultriform process is longer in the specimens from Ñambi, Pacific Colombian and this might be supported by the genetic distance among populations from Ñambi (JDL29286, JDL39286), Buenaventura (MT165) and Darien (Panama), ranging from 3.5% to 4.0%. *Pristimantis condor*, known from the Cordillera del Condor (Ecuador and Peru), is sister to a clade B, containing species distributed fundamentally in lowland upper Amazon basin: *P. conspicillatus*, *P. malkini* and *P. peruvianus* (TG6030) and on eastern foothills of central Andes (*P. buccinator* and *P. citriogaster*).

Clade B also contains the species *Pristimantis sp* SBH2007 which forms a politomy with *P. buccinator*, and sequence divergence among these samples was 1%, indicating that the sample *Pristimantis sp* (SBH2007) is an individual of *P. buccinator*. As has been observed in the studies on terraranas, *P. citriogaster* is the sister species of *P. malkini (*from Sucumbios-Ecuador-QCAZ28296), but the topology shows that *P. malkini* from Mitu-Colombia (RPB489) and from Ecuador are two different lineages, these two "populations" between Ecuador and Colombia show a significant genetic divergences (5%). *Pristimantis bipunctatus*, *P. skydmainos* and a sample identified as a *P. peruvianus* (27169) are clustered together, and are part of the second clade B.

Clearly, there are two different lineages under the name of *P. peruvianus*, we have not seen the sample *P. peruvianus* (27169), and therefore we are going to refer as a "*P. peruvianus*". *Pristimantis vilarsi* is distributed in the upper Amazonian Brazil, Colombia, Venezuela, and Peru and this species is found in regions of a highly eroded sandstone plateau. The populations of *P. vilarsi* from Colombia (San Juan de Arama and Vaupes), and Venezuela forming a politomy, and are sister to a samples identified as *Pristimantis sp* (MT87-MT89) from Tepequem (Roraima, Brazil), with genetic divergences among populations reaching 2.5%. The samples of all this clade are relatively near to the type locality of *P. vilarsi* (Taracuá, Rio Vaupés, and Amazonas, Brazil). We have not seen the individual identified as a *P. memorans* (Barcelos, Amazonas; MT61 and MT64) but comparing the original description of this species with other species placed in the *P. conspicillatus,* it does not fit and we suggest that these individuals are misidentified. The samples from Barcelos are clustered together with the *P. vilarsi* from Colombia and Venezuela, showing genetic divergence of 4.5% with respect to *P. vilarsi* from Colombia. Surprisingly, the sample of *P. vilarsi* from Mitú (RPB475) Vaupes in Colombia is in fact recovered as the sister taxon of *P. zeuctotylus* and thus renders *P. vilarsi* paraphyletic. Samples identified as a *P. fenestratus* (MT118 and MT120) from Ariquemes, Roraima, are the sister taxon of all these species.

The clade C is subdivided in two subclades. Samples of individuals identified as *Pristimantis sp* from Carolina, Maranhon (MT85) and *P. fenestratus* from Para (MT54, MT98 and MUZUSP139440) cluster together. This clade is sister to *P. chiastonotus*, which occurs in northeaster Brazil, Guyana, French Guyana, and Surinam. *Pristimantis fenestratus* from Ariquemes, Rondonia (MT107) and Parecis, Rondonia (MT114 and MT147), collapsed in a politomy, and it is the sister taxon to a clade composed of *P. ventrigranulosus* (Mato Grosso=MT72 and Goias=MT96), who is sister to a clade composed of *Pristimantis sp* from Mato Grosso (MT77 and MT75) and from Goias (MT81). Samples of *P. fenestratus* from Bolivia (MNKA6629-6631; MNCN43031-4109; MHNC3130) are sister to a clade composed of "*P. fenestratus*" from Peru and Bolivia (MNCNDNA9505 and SBH2007; showing genetic divergence of 1%)*, P koehleri*, *P. samaipatae* and samples of *P. fenestratus* (MT111) and *Pristimantis sp* (MT108 and MT46) from Pará and Amazonas. The clade composed of samples identified as *P. fenestratus* from Peru and Bolivia is sister to *P. koehleri*, which occurs in the Andean slopes of Bolivia.

The second subclade is composed of two geographically contrasting clades: a monophyletic group composed of species from the north-east of Brazil (*P. vinhai*, *P. paulodutrai* and *P. ramagii*), and *P. gaigei* known from the biogeographical region of Chocó, from eastern Panamá to Valle del Cauca in Colombia, and in the middle Magdalena valley area and humid areas at the northern of the Cordillera Central (Colombia).

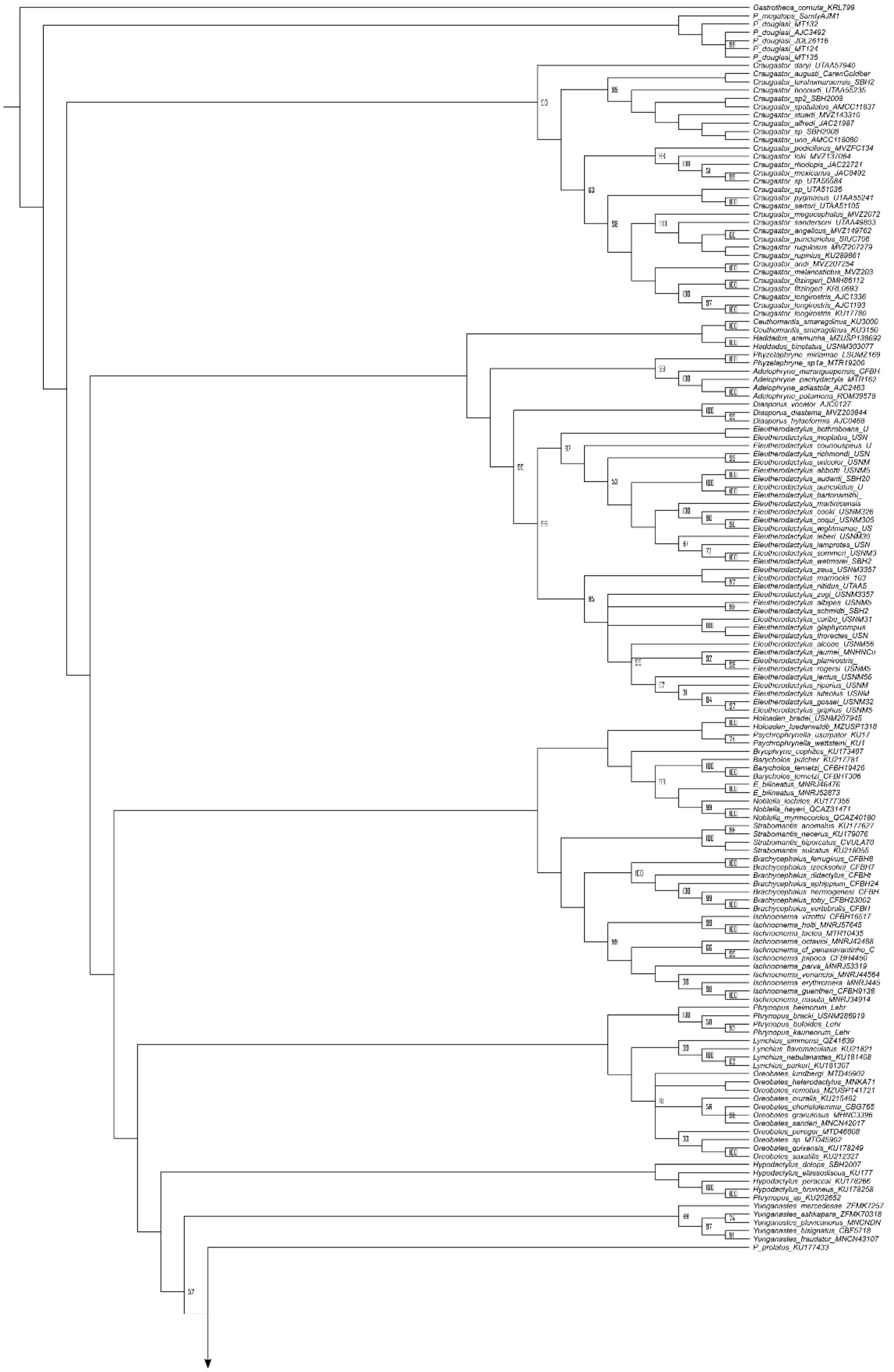

Figure 14. Strict consensus of 38 most parsimonious trees of 76253 steps for a dataset 17635 aligned sites of mitochondrial and nuclear DNA, and 617 terminals included in *Brachycephaloidea* and *Gastrotheca cornuta* (Hylidae) as the root. Numbers in the nodes indicate >50 values of bootstrap support.

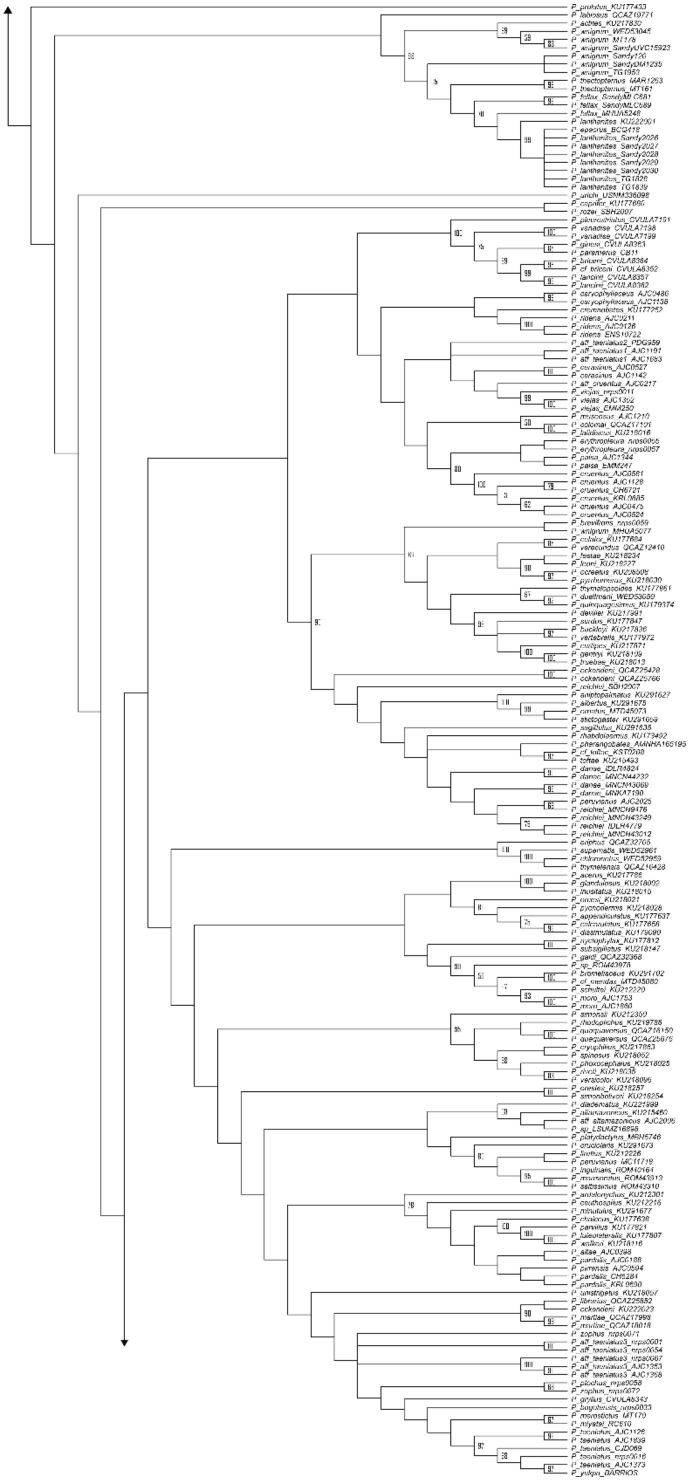

Figure 15. Strict consensus of 38 most parsimonious trees of 76253 steps for a dataset 17635 aligned sites of mitochondrial and nuclear DNA, and 617 terminals included in *Brachycephaloidea* and *Gastrotheca cornuta* (Hylidae) as the root. Numbers in the nodes indicate >50 values of bootstrap support.

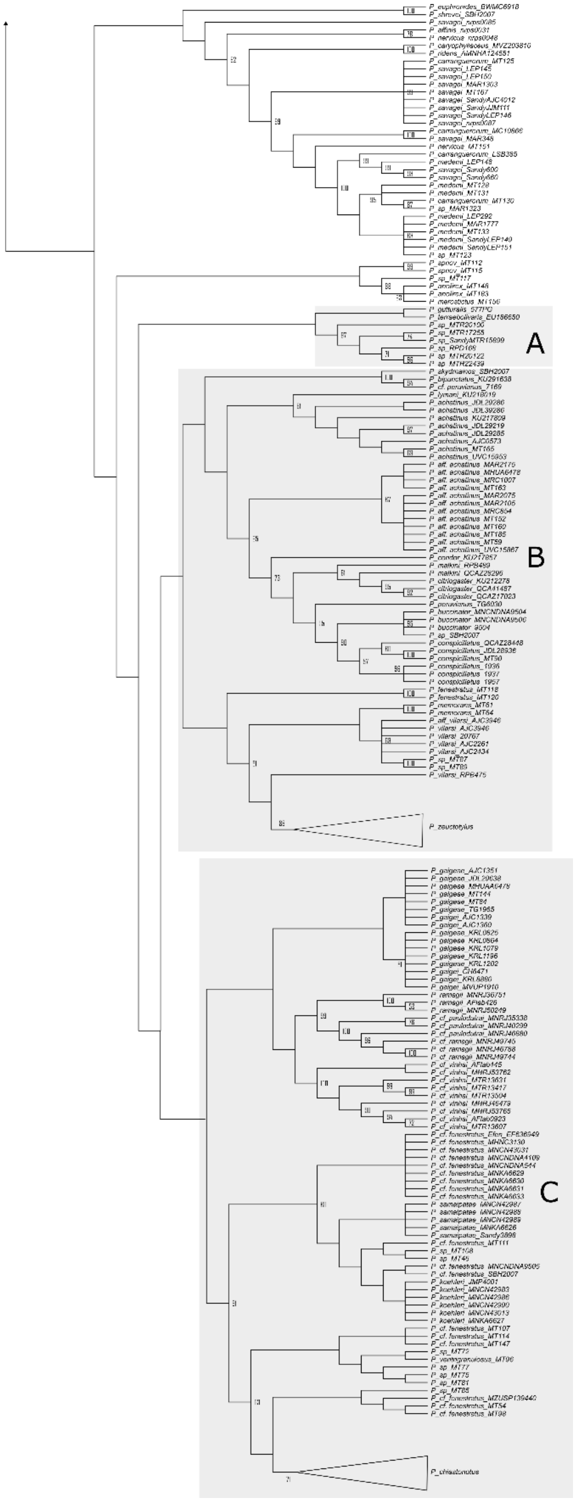

Figure 16. Strict consensus of 38 most parsimonious trees of 76253 steps for a dataset 17635 aligned sites of mitochondrial and nuclear DNA, and 617 terminals included in *Brachycephaloidea* and *Gastrotheca cornuta* (Hylidae) as the root (part3). The letters in

the shadow squares indicate the clades discuted in the text. Numbers in the nodes indicate >50 values of bootstrap support.

The sister clade of *Pristimantis conspicillatus* group

A clade composed of *P. anolirex*, *P. merostictus* and *Pristimantis sp*, which occurs in the north of the Cordillera Oriental of Colombia was found to be a sister taxon of *P. conspicillatus* group. These species are sister to a clade containing *P. nervicus*, *P. carranguerorum, and P. savagei. P. medemi*, and *Pristimantis sp*, species that also occur in the Cordillera Oriental of Colombia.

Parsimony analysis with molecular and morphology data

Parsimony analysis (molecular + morphology) yield the same topology as the parsimony with molecular data tree (Appendix 5). *Pristimantis conspicillatus* group was recovered as a monophyletic and again the clade composed of *P. anolirex*, *P. merostictus* and *Pristimantis sp* is recovered as a sister group. Some species of *Eleutherodactylus*, *Craugastor*, *Haddadus* and *Hypodactylus* are collapsed in a polytomy, making these genera paraphyletic. Morphological characters used in this study do not support the *P. conspicillatus* group monophyly, the analysis does not a find morphological synapomorphy for *P. conspicillatus* group. However the analysis detected two synapomorphies that supports clades within and outside of the *P. conspicillatus* group.

Maximum likelihood analysis

The phylogenetic hypothesis of the *Pristimantis conspicillatus* group

GARLI search under the optimal 2-partition + GTR model identified a single maximum likelihood tree (log likelihood=-332802.8; Figures 17, 18 and 19). The ML analysis recovered the same *P. conspicillatus* group observed with parsimony analysis, which splits into four large clades. Three of these four clade are well supported. In the clade A, *P. gaigei* is sister to all species of the Atlantic forest (*P. paulodutrai*, *P. ramagii*, *P. vinhai* and the clade containing at least five undescribed species). It is sister to a clade B, composed of *P. terraeboliviaris* and *P. gutturalis* that are cluster together and are sister to a clade containing samples from Ariquemes, Roraima (*P. fenestratus*, MT118), Tepequem, Roraima (*Pristimantis sp*, MT87 and MT89), Barcelos, Amazonas (MT61 and MT64), *P. zeuctotylus* and *P. vilarsi*. Again *P. vilarsi* is recovered as a paraphyletic with respect to the samples from Amazonas, Brazil.

The clade C is composed of *P. lymani* and *P. achatinus* (from Pacific cost of Colombia and Ecuador), *P. citriogaster*, *P. malkini*, *P. peruvianus*, *P. buccinator*, *P. conspicillatus*, *P. condor* and *P. achatinus* (from Cordilleras Occidental and Central of Colombia), which cluster together and are the sister taxon of a clade composed of *P. buccinator*, *P. skydmainos* and "*P. peruvianus*". This well−supported analysis show *P. condor* is sister to *P. aff. achatinus*, and *P. malkini* is recovered as monophyletic.

The clade D is the same clade C identified in parsimony analysis without the subclade composed of *P. gaigei* and the species of the Atlantic Forest. The clade composed of samples identified as *P. fenestratus* from Peru and Bolivia, and *Pristimantis* sp from Para and Amazonas (Brazil) is sister to P. *koehleri*.

The sister clade of *Pristimantis conspicillatus* group

ML analysis found that the species from the Cordillera Oriental (*P. anolirex*, *P. affinis*, *P. merostictus*, *P. medemi*, *P. carranguerorum, P. savagei* and samples identified as a *Pristimantis sp*), and species from Choco biogeographic region are clustered together to form the sister clade of *P. conspicillatus* group.

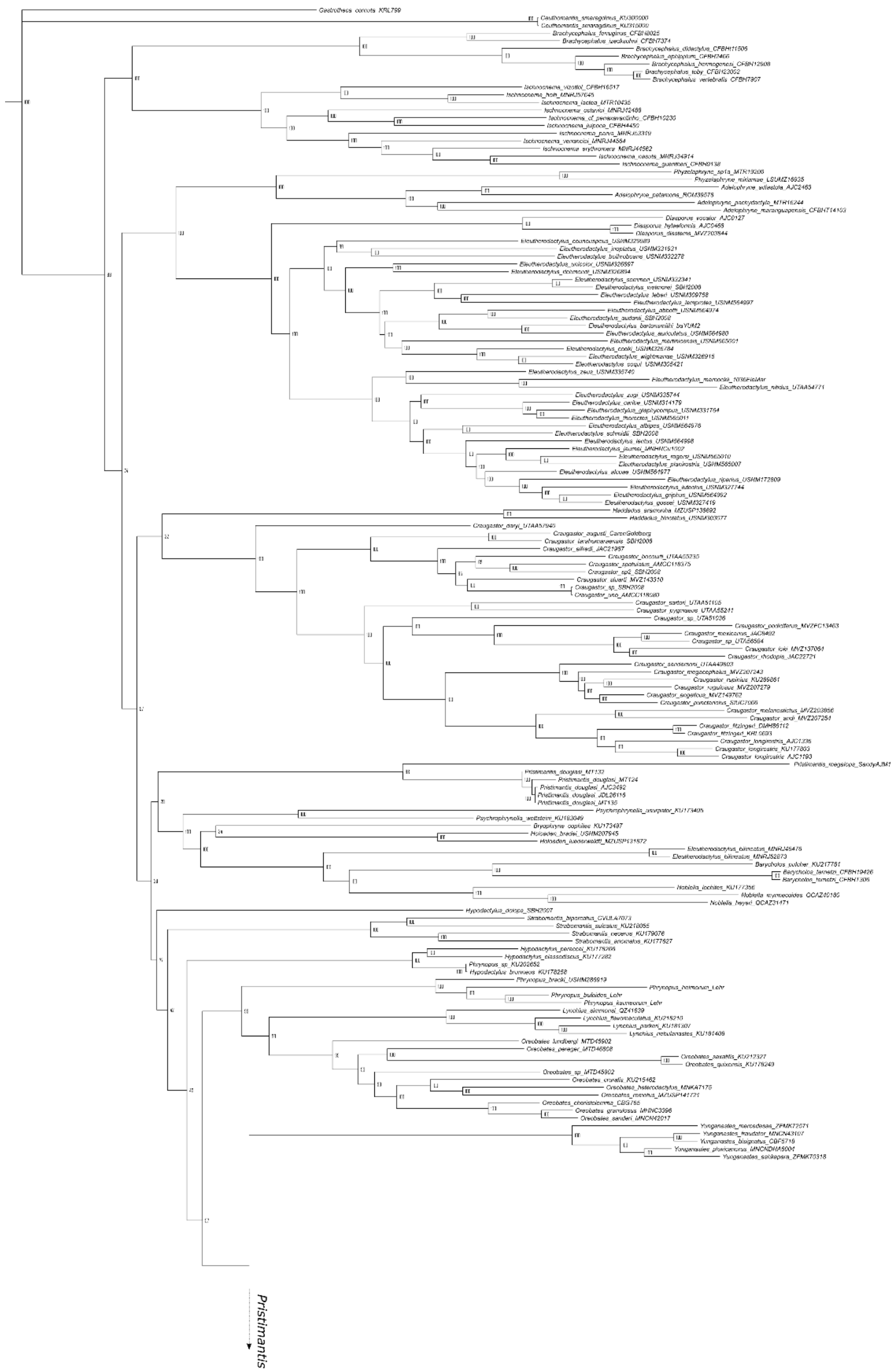

Figure 17. Optimal solution (ln likelihood= -332802.8) for a dataset of 17635 aligned sites of mitochondrial and nuclear DNA, and 617 terminals included in *Brachycephaloidea* and *Gastrotheca cornuta* (Hylidae) as the root (part 1). Assuming mitochondrial and nuclear

partition and the GTR + G substitution model. Numbers in the nodes indicate bootstrap support values.

Figure 18. Optimal solution (ln likelihood= -332802.8) for a dataset of 17635 aligned sites of mitochondrial and nuclear DNA, and 617 terminals included in *Brachycephaloidea* and

Gastrotheca cornuta (Hylidae) as the root (part 2). Assuming mitochondrial and nuclear partition and the GTR + G substitution model. Numbers in the nodes indicate bootstrap support values.

Figure 19. Optimal solution (ln likelihood= -332802.8) for a dataset of 17635 aligned sites of mitochondrial and nuclear DNA, and 617 terminals included in *Brachycephaloidea* and

Gastrotheca cornuta (Hylidae) as the root (part 3). Assuming mitochondrial and nuclear partition and the GTR + G substitution model. Numbers in the nodes indicate bootstrap support values. Letters in shadow rectangles indicate clades discuted in the text.

Bayesian Inference analysis

The phylogenetic hypothesis of the *Pristimantis conspicillatus* group

Bayesian topology was congruent with ML. IB analysis showed a highly supported monophyletic *P. conspicillatus* group (Figures 20, 21, 22 and 23). Bayesian and Ml topologies are more similar to each other than with MP. IB analysis splits *P. conspicillatus* group into four large clades, and these clades are the same that in ML. Each of these four clades is in general well supported. Two differences are between IB and ML. *Pristimantis malkini* is recovered as paraphyletic and *P. koehleri* is sister to *P. fenestratus* form Peru and Bolivia instead of being sister to a clade composed of *P. fenestratus* from Peru and Bolivia and *Pristimantis* sp from Para and Amazonas (Brazil). The sister clade of *P. conspicillatus* group is the same that ML analysis recovered.

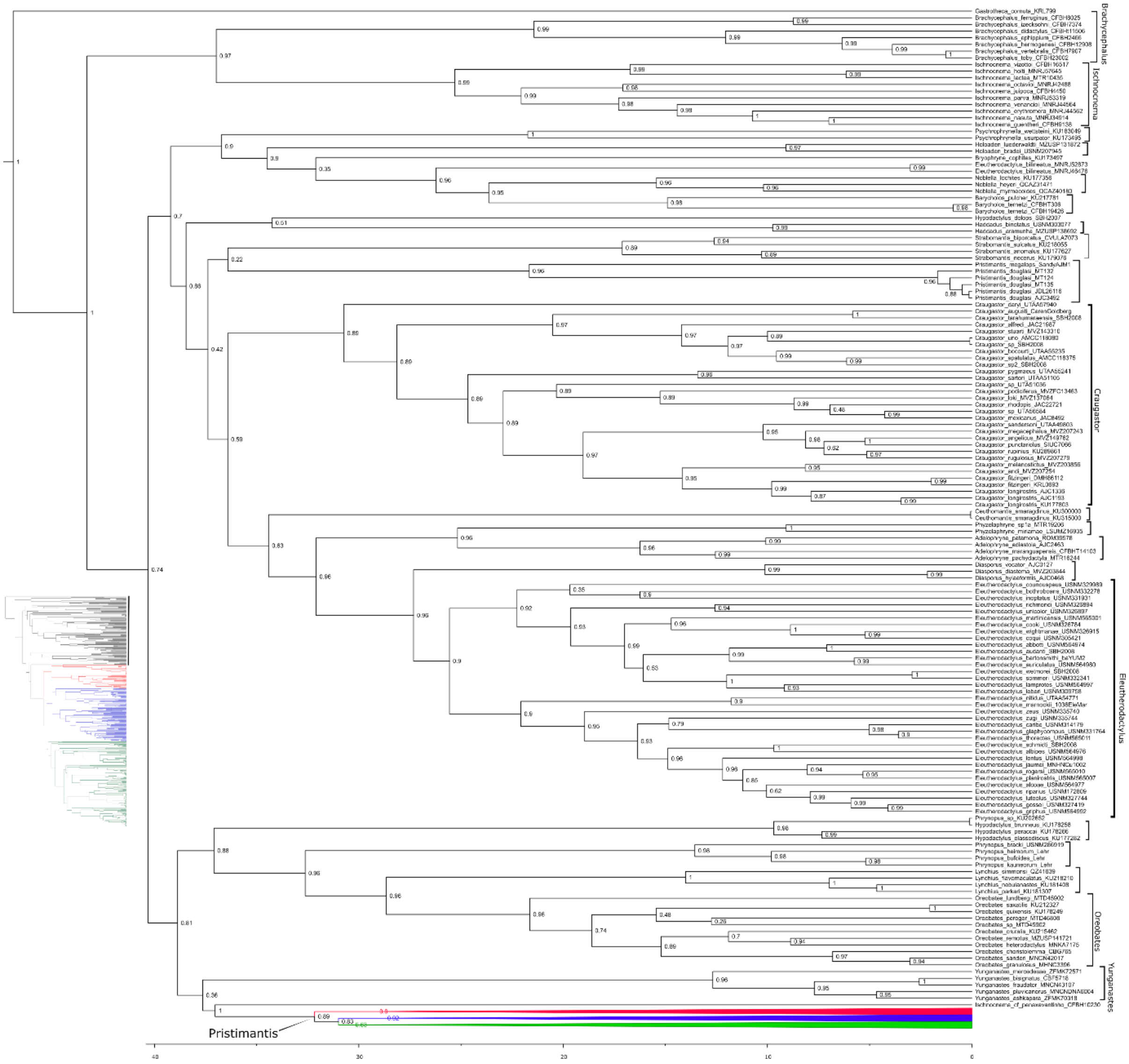

Figure 20. Mitochondrial + nuclear phylogeny of Terarrana. Maximum clade credibility subtree showing the taxa different to *Pristimantis* included in the study. Nodes are labelled with the posterior probability values.

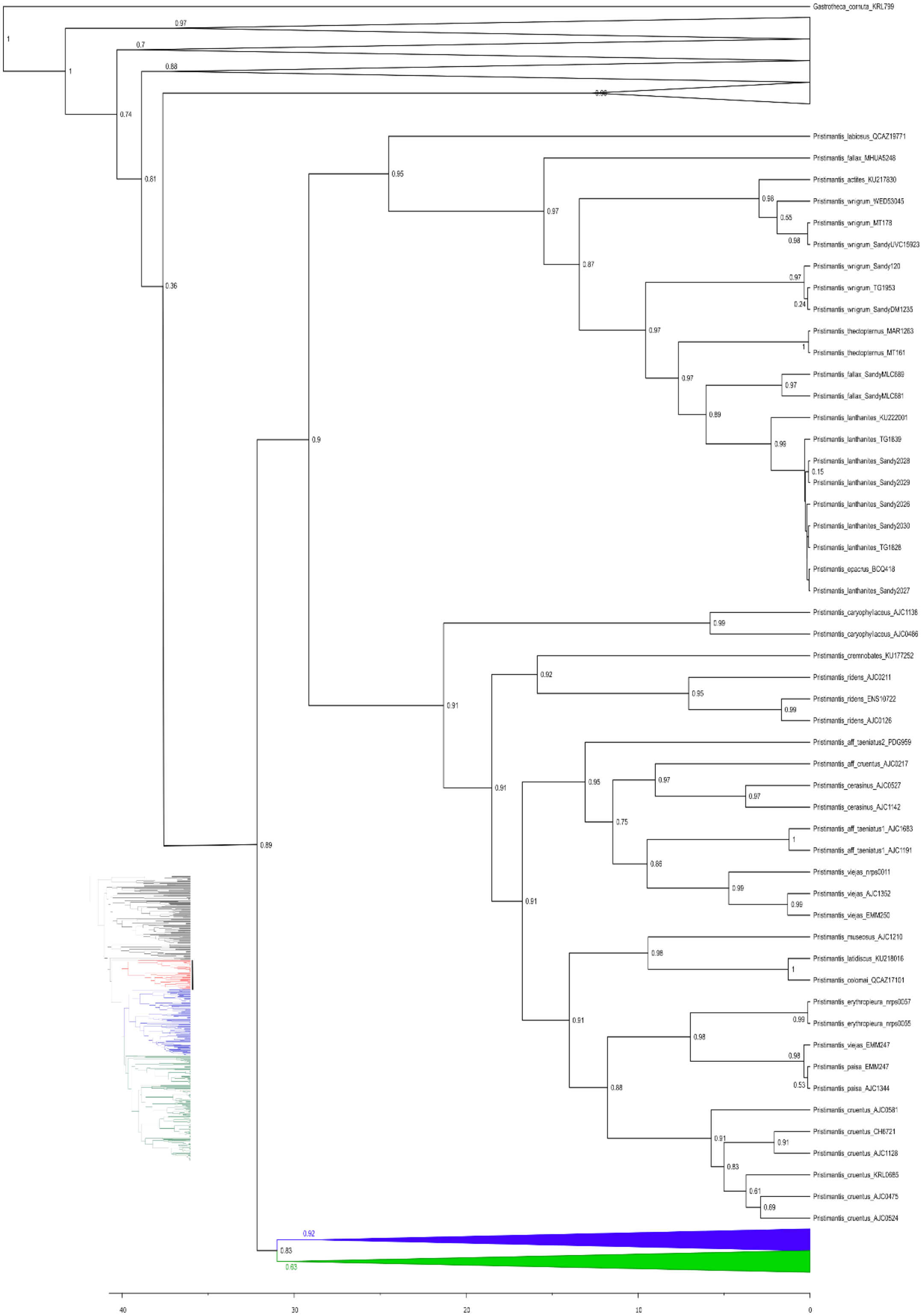

Figure 21. Mitochondrial + nuclear phylogeny of Terarrana. Maximum clade credibility subtree showing the most internal clade of *Pristimantis* as separated in this study. Nodes are labelled with the posterior probability values.

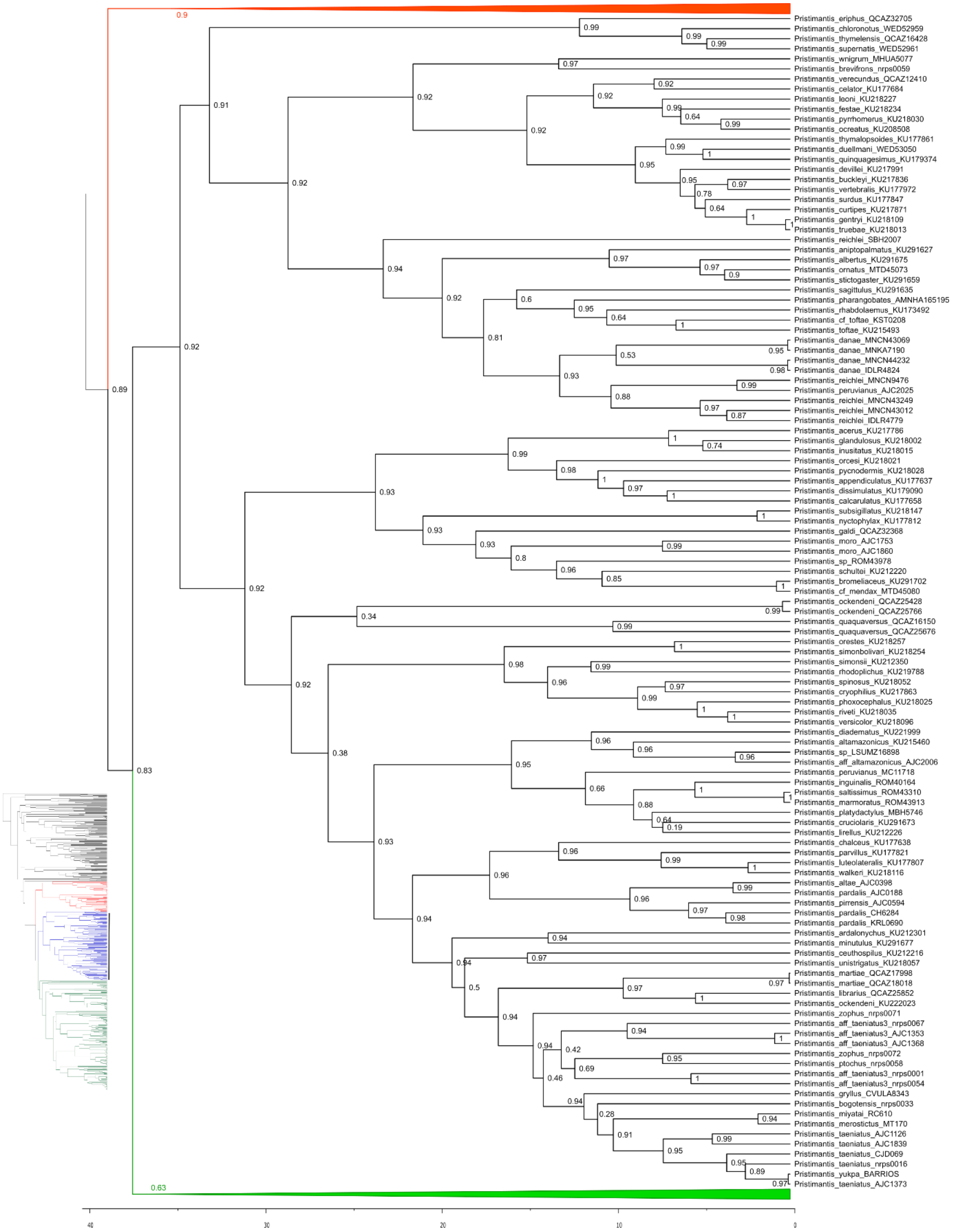

Figure 22. Mitochondrial + nuclear phylogeny of Terarrana. Maximum clade credibility subtree showing one of the two most external clades of *Pristimantis* as separated in this study. Nodes are labelled with the posterior probability values.

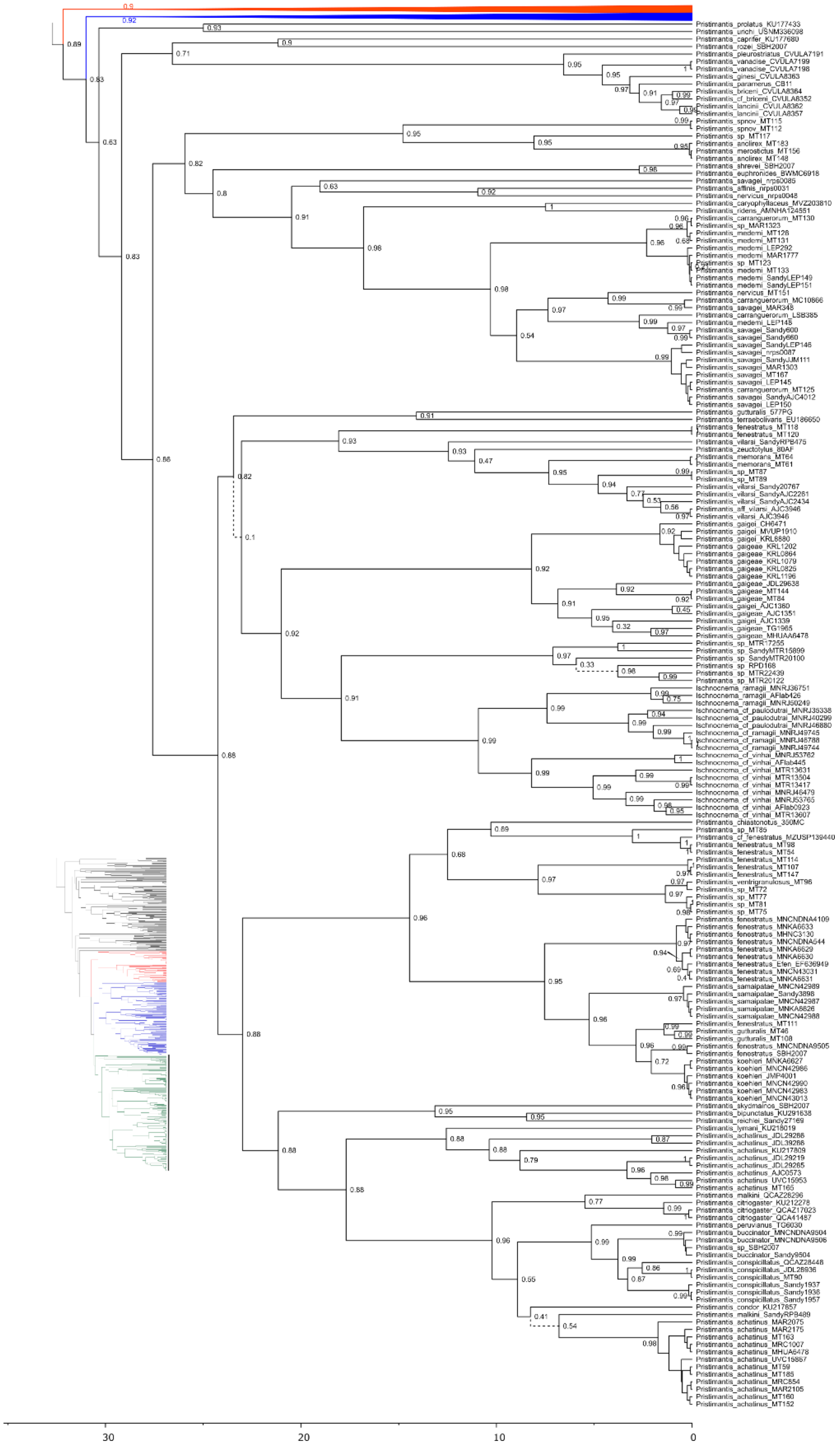

Figure 23. Mitochondrial + nuclear phylogeny of Terarrana. Maximum clade credibility subtree showing one of the two most external clades of Pristimantis as separated in this study. Nodes are labelled with the posterior probability values

Evolution of Characters

We have optimized and examined the evolution of 32 characters on our condensed version of the phylogenetic hypothesis of MP and ML. The following analysis of character evolution should be interpreted in light of two caveats: First, data were taken from either personal observations, field notes and photographs, or published sources and second, there are extensive missing data for several of the characters, especially in the outgroup species, and for the undescribed species from Brazil placed in *P. conspicillatus* group. We analyse below, and, although the most parsimonious optimization often allows unambiguous prediction of unknown states, it is possible that future discoveries will overturn some predictions and favour alternatives.

External Morphology

The optimization of the presence/absence of the any type of texture on the skin of the venter (Character 1) (Appendix 4), shows that the ancestral state for *P. conspicillatus* group is venter with texture (granular, coarsely granular, aerolate). The absence of texture on the belly have evolved six times in the *P. conspicillatus* group. The absence of texture on belly occurs in all the species of the clade composed of: *P. lymani*, *P. achatinus*, "*P. achatinus*", *P. condor*, *P. malkini*, *P. citriogaster*, *P. buccinator*, and *P. conspicillatus.* It is remarkable that the presence of smooth skin of venter has an independent origin in *P. caprifer*, which was allocated into the *P. conspicillatus* group by Lynch & Duellman (1997) and now is not assignable to any *Pristimantis* species group *sensu* Padial *et al.* (2014). The presence of smooth skin of the venter occurs within Brachycephaloidea in the genera: *Craugastor*, *Ischnocnema* and *Oreobates*.

We must clarify that this character applies to the texture of the belly and texture of the dorsolateral flanks was not taken into account. This has probably been an error in terms of description of venter texture. Sometimes there are granulations of lower flanks encroach on posterior parts of the venter, and this observation can be seen as a belly with texture. Furthermore, the type of skin of venter should be treated with care, in most cases this feature is prone to preservation artefacts. As Lynch & Duellman (1997) noted, if the texture of the skin of the venter is granular, it is most likely affected by preservation fluids and techniques; thus, a dead specimen that is later preserved may appear to have smooth skin on the belly, even under high magnification.

Following with the texture of the skin, the optimization of the tubercle on the upper eyelid (character; Appendix 4) shows that the presence of round tubercle has evolve twice independently and presence of subconical tubercle once. The optimization of ornamentations on the heel (character 3), shows that the ancestral state for *P. conspicillatus* is absence of ornamentation. The presence of an ornamentation on the heel has one independent origin in *P. conspicillatus* group. Most of the *P. conspicillatus* species have the skin of the upper eyelid, the tarsus and the heel with the same texture. The presence of supra anal warts (character 4) has an independent origin in the *P. conspicillatus* group. Lynch & Suárez-Mayorga (2000) proposed that the conical warts represented a synapomorphy for the *P. conspicillatus* group, linking: *P. epacrus*, *P. fallax*, *P. gutturalis* and *P. lanthanites*. Currently the only species outside of this putative assembly proposed by Lynch & Suárez-Mayorga (2000) is *P. gutturalis* which does belong to the *P. conspicillatus* group.

The absence and the kind of dorsolateral fold (character 5; Appendix 4), has different levels of interpretation according to the phylogenetic inferences, as optimized. The optimization under parsimony shows that the ancestral state is dorsolateral folds anterior, incomplete, and implies that this condition has been lost three times. While the optimization under ML shows that the ancestral state is absence of dorsolateral folds, and implies that this condition has been lost three times. Nonetheless, some patterns can be distinguished, the clade composed of *P. lymani*, *P. achatinus*, *P. condor*, "*P. aff achatinus*", *P. conspicillatus* and *P. buccinator* have complete dorsolateral folds, except for *P. citriogaster* and *P. malkini* which are sister species and share absence of dorsolateral folds. Probably, this character can be informative to some clades within the *P. conspicillatus* group, but much more information is needed.

The optimization of tympanum size (character 7) implies that the presence of a large tympanum (tympanum sizes passes the lower eyelid and reaches the labial commissure) is observed in all the *P. conspicillatus* species group studied, but this character was not yet detected as a synapomorphy. Tympanum size may be particularly useful in the characterization of *P. conspicillatus* group.

The optimization of vocal slits in males (character 8; Appendix 4) shows that it was lost two times in *P. lymani* and *P. gaigei* within the *P. conspicillatus* group. Currently no species group or clade inside Brachycephaloidea has been diagnosed by the absence of secondary sex character. However, in the clade of eight species of *P. devillei* group (*P. devillei*, *P. buckleyi*, *P. curtipes*, *P. duellmani, P. quinquagesimus*, *P. surdus*, *P. truebae* and *P. thymalopsoides*), the absence of vocal slits is observed in each of the species; this character could support the diagnosis of a clade.

The taxonomic distribution of the relative length of the finger (character 9; Appendix 4), indicates with the information available that within *Pristimantis*, the first finger longer than second occurs in the *P. conspicillatus* species group (except for *P. vinhai*). The first finger longer than second occurs within

Brachycephaloidea in the genera: *Craugastor, Strabomantis,* and *Oreobates* (data not included in our matrix). We noted no correspondence between the relative lengths of the finger I and II described in the original descriptions and the respective specimens. The species that were originally described with the first finger longer than second (*P. actites*, *P. caprifer*, *P. fallax*, *P. lanthanites* and *P. w-nigrum*), the second finger is slightly longer than the first. With cleared material in populations of *P. w-nigrum*, we corroborate that second finger is longer than the first, but this relation in the length is different to other *Pristimantis* species (*P. carranguerorum*, *P. medemi* and *P. savagei*) where the second finger is much longer (not slight longer than the first). Therefore, we are aware that within the relative lengths of the finger I and II, it is possible to propose more character states.

Duellman & Lehr (2009) observed that ontogenetic changes occur in the relative length of the fingers in at least some species (*Hypodactylus lucida*, Lehr, pers. comm.) in which adults have Finger I longer than finger II. In juveniles, finger I is shorter than finger II (Duellman & Lehr, 2009). We did not observe any ontogenetic changes in the relative length of fingers I and II. We did not take into consideration another possible state, finger I and II equal in length. We are not aware of a terrarana species having this condition.

Shank size (character 14; Appendix 4), has different levels of interpretation according to the optimized phylogenetic inferences. Under parsimony, the ancestral state for *P. conspicillatus* group is ambiguous, while under ML analysis the ancestral sate is medium shank size. Nonetheless, some patterns can be distinguished, the clade composed of *P. lymani*, *P. achatinus*, *P. condor*, "*P. aff achatinus*", *P. conspicillatus, P. condor, P. citriogaster*, *P. malkini*, *P. skydmainos* and *P. bipunctatus* have large shanks.

Cranial Osteology

The optimization of the characters: 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 29, 30, 31, 32 (Appendix 4), show that the ancestral state is the same one for the genus *Pristimantis* and for *P. conspicillatus* species group. The following states of characters are observed in all *P. conspicillatus* species group for which osteological information was available. The alary process is directed dorsally (character 16), the nasals bones are separated medially but half or more of its length is in contact (character 17); nasals and frontoparietals are not in contact (character 18); and fusion between frontoparietals and the prootics absent (character 19); cranial crest absent (character 20); frontoparietal fontanelle anteromedially not exposed (character 21); without ornamentation along the zygomatic and otic rami of the squamosal (character 22); otic ramus of the squamosal shorter than zygomatic ramus (character 23) and the length of the otic ramus of the squamosal does not extend beyond the crista parotica dorsally (character 25); triangular dentigerous process of the vomer (character 26), moderate sized sphenethmoid (character 29); the cultriform process reaches the palatines (character 30); the anterior ramus of the pterygoid does not reach the anterior edge of frontoparietals (character 31) and, fusion of the sacral an the eight presacral vertebrae absent (32)

Current evidence indicates that at least three independent origins of the alary process directed posterodorsally in *Tachiromantis* (*Tachiromantis douglasi* and "*P. megalops*"), in *Strabomantis* (*S. sulcatus*) and in *Pristimantis* (*P. buckleyi*). In our data set, the alary process directed posterodorsally is observed in *P. delicatus*, *P. insignitus*, and *P. ruthveni* (species without sequence available to optimize). Lynch & Ruiz-Carranza (1985) and Lynch (1996) describe the alary process in some species of the Sierra Nevada de Santa Marta (SNSM) as slightly posterodorsally. Here we consider that *P. cristinae*, *P. delicatus*, *P. insignitus*, *P. megalops*, *P. ruthveni* and *P. sanctamartae* have the alary process directed posterodorsally. After we revisited the specimens we saw that the slopes of the alary process is significant enough to be describe as a posterodorsal. In our data set, the nasals and frontoparietals in contact is observed in the genus *Craugastor* and *Strabomantis,* and with this information on the hypothesis of phylogeny, we suggest that contact between nasal and frontoparietals has evolved independently twice. Considering published information on the cranial osteology, the optimization of separation between the nasal bones, shows that completely separate nasals have evolved at least three times. With respect to the fusion between frontoparietals and the prootic, not all the 93 species with fusion are in topologies and these are necessary to help interpret the evolution of fusion between frontoparietals and the prootic. With the incomplete information on the hypothesis of phylogeny the optimization indicates that fusion between frontoparietal and the prootics have evolved at least twice in *Eleutherodactylus* and *Pristimantis* and once in *Tachiromantis*.

Cranial crests occurs in a variety of terraranas, such as: the *Strabomantis biporcatus* and *S. cornutus* groups, the *P. ridens* and *P. galdi* groups and in the new clade. Not all these species are in topologies and these are necessary to help interpret the evolution of cranial crest. With the incomplete information on the hypothesis of phylogeny, the optimization shows that cranial crest have evolved at least once in *Strabomantis* and in the new clade, and in *Pristimantis* at least four times. Surprisingly, in the clade of eight species of *P. devillei* group, where the absence of vocal slits is observed in each of the species, presence of cranial crest also support this small clades. The presence of cranial crests is often viewed as a convergence.

Guayasamin (2004) hypothesized that the presence of an exposed frontoparietal fontanelle is homologous in all species of the *Pristimantis orcesi* group and also suggested that the exposed frontoparietal fontanelle is a derived character with respect to the rest of *Pristimantis*, supporting the monophyly of the *P. orcesi* group. However, when this character was optimized, two species assigned in the *P. orcesi* group appear to be unrelated and shared exposed fontanelle. Furthermore, Guayasamin (2004) discussed the presence of an exposed frontoparietal fontanelle in species of the genus *Eleutherodactylus*, *Ischonocnema, Oreobates*, *Pristimantis* and *Yunganastes*. Lynch *et al.* (1996) stated that the exposure of the frontoparietal fontanelle in the *P. orcesi* group probably was not a derived character. The frontoparietal fontanelle is exposed in juveniles of terraranas, but this condition is retained in the adults of relatively few genera (Lynch, 1971). Padial *et al.* (2007) reported that all known species described and assigned to the *Yunganastes* genus exhibit an exposed frontoparietal fontanelle, but they did not consider the presence of this structure as a valid synapomorphy of *Yunganastes*. The present study confirms that a fontanelle exposed has evolved multiple times. A study of the developmental pathway of this structure would reveal an independent origin for the different groups where it appears (Padial *et al.*, 2007).

In lateral profile, several variations on the squamosal are apparent in the lengths and sizes of the zygomatic and otic rami (Lynch, 1971). For the species sampled, the zygomatic ramus does not articulate with other bones of the skull. Among terraranas, the zygomatic ramus terminates in a variety of forms which can be described as: robust, elongate, long, short and poorly differentiated. We observed more variation in the shape of the zygomatic ramus in the outgroup species, for instance, *P. galdi* and *P. quinquagesimus* have a shorter zygomatic ramus, but it is less elongate and more robust, and in *P. orestes, where* the zygomatic rami is inconspicuous and poorly differentiated. Here we are interested in the relative length between the zygomatic and otic rami of the squamosal. We take two different kinds of evidence to argue that (within terraranas) the growth of these two structures of the squamosal is independent. The otic ramus is medially expanded that broadly articulates with the dorsal portion of the cristae paroticae (Lynch, 1971). The origin and growth of these rami occurs during different stages of development in *Eleutherodactylus coqui*, at stage 13, the otic ramus extends posteriorly alongside the auditory capsule, while at stage 15 a short zygomatic ramus appears anteriorly, delimiting the otic and ventral rami (Hanken *et al.*, 1992). The optimization of the length of the zygomatic and an otic rami of squamosal implies that otic ramus of the squamosal shorter than zygomatic ramus has at least three independent origins in terraranas, in the new clade, in *Craugastor* and in *Strabomantis*.

We observed a large sphenethmoid, which extends past the anterior edge of the nasals in *P. megalops*, a member of the new clade. Lynch (1989; 2003) proposed a large sphenethmoid as a synapomorphy linking the species now assigned to the genus *Hypodactylus (H. adercus, H. latens* and *H. nigrovittatus*) *sensu* Hedges *et al.* (2008). Although these species have a large sphenethmoid extending to a point anterior to the nasals, we emphasize that the nasals in these species appear to be small. The taxonomic distribution of large sphenethmoid suggest at least two independent origins. Further, studies will determines whether if large sphenethmoid is homologous in the new clade and in *Hypodactylus*.

Lynch (1973: 166-167) argued that eight presacral vertebrae were to be observed in all frogs except the ascaphiids (nine presacral) and in some genera of bufonids and possibly in paleobatrachids (seven presacral). The reports of only 5 or 6 presacral by Kluge & Farris (1969) and Trueb (1973) represents confusions derived from not counting vertebrae fused with the sacrum (or the fusion of the two vertebrae). Fusion between adjacent vertebrae are uncommon in frogs but originates by two means: adventitious fusion (detected by observing that the fusion is only an occasional variant) and fusion having a genetic basis (detected as the norm in some species). Non adventitious fusion of the first two vertebrae is known for *Atelopus* and some myobatrachids (Lynch, 1971). Non adventitious fusion of the eighth vertebrae and the sacrum is known for some ranoids (in the

Lithobates palmipes group, Lynch 1965; Hillis & De Sá, 1988) and in the ptychaenids of the genera *Hildebrandtia* and *Ptycadena* (Clark, 1982).

Lynch (1996) reported the fusion of the 8th vertebrae and the sacrum in two species of Colombia *Pristimantis* of the *P. galdi* group and Lynch (2001) reported the same fusion in all species assigned to the *Craugastor gollmeri* group from Middle America. We noticed that some other species of *Pristimantis* from the Sierra Nevada de Santa Marta also have eighth vertebra and the sacrum fused: *P. carmelitae*, *P. cristinae*, *P. insignitus*, *P. megalops*, *P. ruthveni* and *P. sanctamartae)*. Lynch (1996) reported fusion of the eighth presacral vertebrae with the sacrum in *Tachiromantis douglasi* (northern Cordillera Oriental) linking this species with *P. delicatus* of the Sierra Nevada de Santa Marta (SNSM) who shared the fusion, and this result has been taken as a evidence by J. D Lynch for the connection between the isolated frogs fauna of the SNSM and any other geographic unit, the Cordillera Oriental de Colombia. The same fusion is present in another species of *Pristimantis* that has not been described, found in the Serranía of Perijá but no other species in the genus known from the Cordillera de Merida de Venezuela or the Cordillera Oriental of Colombia exhibits the fusion of these two vertebrae. We suggest, that fusion of the 8th vertebrae and the sacrum has at least two independent origins.

The optimization of the shape of the squamosal ventral ramus indicates that the curve ventral ramus of squamosal only occurs in some species of *P. conspicillatus* group, shared by: *P. achatinus*, *P. condor*, *P. conspicillatus*, *P. fenestratus*, *P. malkini*, *P. peruvianus* and *P. vilarsi.* However, this “curved condition” has to be studied in depth, we suggest that the shape of the ventral ramus of squamosal can be helpful in the diagnosis of the *P. conspicillatus* group.

The optimization of the size of palatines (character 28), indicates that short palatines appears as derivate states in many clades of Brachycephaloidea. It is remarkable that short palatines occurs in a clade of *P. conspicillatus* group with a large disjunction, between northern South America (*P. gaigei*) and Atlantic Forest (*P. paulodutrai* and *P. ramagii*). Short palatines is a synapomorphy for this clade of four species.

Discussion

Phylogenetic relationship of *P. conspicillatus* group

The MP, ML and IB analysis show congruent results, in each analysis the *P. conspicillatus* group is recovered, although with different support in each analysis. The polytomy formed in the outgroup in parsimony analysis (molecular + morphology), is partially explained by the missing data in the outgroup terminals and there is some incompatibility between molecular and morphological characters. The main differences are among the relationships between the clades within *P. conspicillatus* group under MP. The most remarkable differences resulted involves the placement of *P. gutturalis* and *P. terraeboliviaris*. In ML and IB analysis, *P. gutturalis* and *P. terraeboliviaris* are sister to a clade composing mainly by *P. vilarsi* and *P. zeuctotylus*. This clade is sister to a clade composing of Atlantic Forest species and *P. gaigei*.

The three analysis are congruent and indicate that the *Pristimantis conspicillatus* group is not monophyletic under the definitions of Lynch & Duellman (1997) or Hedges *et al.* (2008) and *Padial et al.* (2004) (Figure 14), therefore the *Pristimantis conspicillatus* group should be redefined. The hypothesis of a monophyletic *Pristimantis conspicillatus* group includes the following twenty three species: *P. achatinus*, *P. bipunctatus, P. buccinator, P. citriogaster, P. chiastonotus, P. condor, P. conspicillatus, P. koehleri, P. fenestratus, P. gaigei, P. gutturalis, P. lymani, P. malkini, P. paulodutrai, P. peruvianus, P. ramagii, P. samaipatae, P. skydmainos, P. terraeboliviaris, P, ventrigranulosus, P. vilarsi, P. vinhai, and P. zeuctotylus.*

In this study, it is clear that *P. peruvianus* and *P. conspicillatus* are two different species. *Pristimantis conspicillatus* and *P. peruvianus* were species previously considered to be only weakly separable and possibly conspecific (Lynch, 1975). The species *P. peruvianus* was removed from the synonymy of *P. conspicillatus* by Lynch (1980). It is evident that the differences in body size and coloration pattern argued by Lynch (1980) are useful to separate these two species. We also observed differences at level of skull; *P. peruvianus* has the dentigerous process "anterior" (does not reach the level of the palatines while they are posterior in *P. conspicillatus*). The records of *P. peruvianus* of the piedmont of Colombia (Pepino, Putumayo), listed by Lynch & Duellman (1980), correspond to an undescribed species, very different from *P. peruvianus* and closer to *P. lanthanites*. It is remains to known the limits of distribution for each species, given that these have large distributional ranges in the Amazon basin of Colombia, Ecuador, Bolivia, Peru and Brazil, and we only studied populations of *P. conspicillatus* and *P. peruvianus* from Colombia. More sampling effort along large areas that are still remain unexplored accompanied by rigorous taxonomic studies would help to understand the distribution of these two species.

Pristimantis conspicillatus has been more closely related to *P. condor* (Pinto-Sánchez *et al.*, 2012; *Padial et al.*, 2014) but in this study *P. conspicillatus* is sister to *P. buccinator* and, *P. peruvianus* (from Leticia) is the sister taxon of these two species. Furthermore, in previous studies, *P. skydmainos* was sister to *P. bipunctatus*. Here *P. bipunctatus* and "*P. peruvianus*" (27169) cluster together and *P. skydmainos* is the sister taxon. The phylogeny hypothesis of *P. conspicillatus* group confirm that *P. vilarsi* and *P. zeuctotylus* are two different distinct lineages, clearing the doubts of Barrio-Amorós & Molina (2006) about the identity of these two species. We considered that presence/absence of the tarsal fold not the only feature that differentiates *P. vilarsi* from *P. zeuctotylus*. The presence of palmar tubercle shape (round) is a valid feature to distinguish species. The shape of palmar tubercle can be changed according to the method of fixing. *Pristimantis chiastonotus* which occurs at low elevations forest in north-eastern Brazil, Guyana, French Guyana and Surinam is sister to a clade conformed of species distributed in Para and Maranhão, *Pristimantis zeuctotylus* also occurs in Guyana is closely related with species that occur in upper Amazonian Brazil, Colombia and Venezuela. In the previous study of terraranas, *P. chiastonus* was more related to *P. fenestratus*, *P. koehleri* and *P. samaipatae* (Pinto-Sánchez *et al.,* 2012; Padial *et al.*, 2014).

Padial & De la Riva (2009) suggested a close relationship between *P. fenestratus* (from Peru and Bolivia) and *P. koehleri,* postulating these two as a sister species. The genetic divergence is considered low between these two species (around 3%), however quantitative characters, bioacustic and reciprocal monophyly, support the independence of these two species (Padial & De la Riva, 2009). In this study, the relationships between these two species are different, *P. koehleri* is sister to a samples from Pará and Amazonas, Brazil (M111, MT108 and MT146), with genetic divergence ranged from 2.5% to 3.0%, and they are sister of *P. samaipatae* and, *P. fenestratus* from Bolivia as the sister taxon of all this clade.

This study found that a clade composing species from the Cordillera Oriental de Colombia are the sister taxon of *P. conspicillatus* group, although with low support in MP. In ML and IB analysis the sister taxon of *P. conspicillatus* group is composed of species formerly assigned to *P. conspicillatus* groups as a *P. carranguerorum*, *P. medemi* and *P. savagei*. Previuos studies in terrarana recovered *P. peruvianus* group as the sister taxon of *P. conspicillatus* group. *Pristimantis carranguerorum*, *P. medemi* and *P. savagei* are distributed in the eastern Andean slopes of the Cordillera Oriental of Colombia. *Pristimantis savagei* was first described from the foothills of the Serranía de la Macarena (Pyburn & Lynch, 1981) but was subsequently found to occupy the eastern Andean slopes of the Cordillera Oriental, where it is more common, rarely descending below 1000 m (Lynch, 1994). *Pristimantis carranguerorum* and *P. medemi* are known from the forests in the piedmont and onto the slopes in the Cordillera Oriental in the departments of Boyacá, Cundinamarca and Meta, but *P. carranguerorum* is restricted to the north of the Cordillera Oriental (Boyacá) and has a higher altitudinal distribution than *P. medemi* (Table 3). In the topology, these three species are clustered with *P. nervicus* and *P. affinis*, which also occur in the departments of Cundinamarca and Meta but at higher elevations above 2600 m. This small clade shares the following external morphology: 1) Narrow heads and long snouts; 2) Skin of dorsum shagreen, skin of venter weakly aerolate; 3) tympanum superficial, but does not reach the labial commissure; 4) first finger much shorter than second; 5) toes lacking webbing, and 6) toe V much longer than III, where toe V reaches about or more than half way between the distal and penultimate subarticular tubercle on toe IV. We suggest that other species such as *P. mnionaetes* might be placed in this clade. Lynch (1998) in the original publication of *P. mnionaetes* postulated this species as sister species of *P. nervicus*. Before that, Lynch (1994) suggest that *P. nervicus* and *P. ginesi* from paramos of Merida, Venezuela, might be closely related. In this study, *P. ginesi* is clustered together with other species of cloud forest and paramos from Merida. *Pristimantis ridens* and *P. caryophyllaceus* occur in lowlands to lower montane in Central America and Choco region. We do not have morphological evidence to support the position of *P. caryophyllaceus* and *P. ridens* within the clade of species of the Cordillera Oriental and we have some doubts about the position of these two species in the clade.

Characterization of the *Pristimantis conspicillatus* group.

On the basis of our phylogenetic hypothesis, we redefine *P. conspicillatus* group. We have not yet found a morphological synapomorphy for the *Pristimantis conspicillatus* group. The characters evaluated in this study were not adequate to demonstrate their status as a natural group. Therefore, the *Pristimantis conspicillatus* species group needs more morphological studies. We provide a characterization, a set of morphological characters states that when combined act as a group of exclusive features to define the *P. conspicillatus* species. This set of features recognizes the species of *P. conspicillatus* group, but we must take into account that historical groups must be supported in terms of evidence, and we have not yet found a shared derived character for the *Pristimantis conspicillatus* group. The results observed in the optimization of characters helps and supports the choice of these characters.

The Pristimantis conspicillatus group includes species with the following combination of characters: 1) Frogs with narrow heads and long snouts; 2) Skin of venter smooth to weakly aerolate but not coarsely aerolate or granular; 3) skin of dorsum uniformly and finely granular (shagreen), without having a pronounced ornamentation (conical or subconical tubercles, on upper eyelid (except for *P. gutturalis*) and heel (except for *P. skydmainos);* 4) Tympanic membrane and annulus present, with a large tympanum, where the tympanum reaches the labial commissure; 5) First finger longer than second (except for *P. vinhai*); Toe V slightly longer than toe III (Toe V and III, does not extend beyond the penultimate subarticular tubercle on toe IV) (except for *P. paulodutrai*, *P. ramagii* and *P. vinhai*).

All of the studied species of *P. conspicillatus* group shared the following characteristics in cranial osteology: The alary process directed dorsally; large nasals in medial contact, the nasals cover much of the nasal capsule and are expanded anteriorly, the distance between the nasals and the premaxilla is reduced, the length of the alary process does not fit within the space between the nasals and the alary process, the anterior border of the nasals is usually convex or nearly straight; contact between the nasals and the frontoparietals was not observed in the *P. conspicillatus* species group, the separation between these two bones is evident; nasals and frontoparietals "compact" (here we use this word to refer to the nasal and frontoparietal have an almost equal width along its length); frontoparietals not fused to the prootics; skull smooth, lacking cranial crests or any type of ornamentation on the nasals, frontoparietals, and along the dorsal edge of the squamosal; the otic ramus of the squamosal is longer than zygomatic, and does not extend beyond the crista parotica posteriorly. All these characteristics mentioned above are shared but are not exclusive to the members of *P. conspicillatus* group.

The optimization of characters helped us to visualize features that are used to characterized clades within *P. conspicillatus* group. For example, the clade composed of *P. lymani*, *P. achatinus*, *P. lymani*, *P. citriogaster*, *P. condor*, *P. malkini*, *P. aff achatinus* and *P. peruvianus* has the skin of venter smooth, complete dorsolateral folds (absent in *P. malkini* and *P. citriogaster* which are sister species), tubercle on heel absent, large shank, first finger longer than second and Toe V slightly longer than toe III (Toe V and III, does not extend beyond the penultimate subarticular tubercle on toe IV).

Short palatines (the distance between the palatines is equal or greater than half the length of one of the palatines) is a synapomorphy linking two geographically contrasting clade from Atlantic forest (*P. ramagii*, *P. paulodutrai* and *P. vinhai*) and a species from mid-Magdalena valley and Choco in Colombia (*P. gaigei*). Lynch & Duellman (1980; 1997) assigned *P. gaigei* to *P. conspicillatus* group despite having little resemblance to other species in the group, this species has a first finger much longer (much longer than other *P. conspicillatus* species group), tympanum round extremely large and prominent subarticular tubercles. *Pristimantis gaigei* is sister to clade of *P. vinhai*, *P. paulodutrai* and *P. ramagii*, and these three species share the following characteristics: Narrow heads and long snouts; skin of dorsum shagreen, skin of venter aerolate; tympanum large; toe V reaches about or more than half way between the distal and penultimate subarticular tubercle on toe IV.

According to this new definition and following only the external character states, we here propose that *P. dundeei* distributed in the Brazilian Cerrado belongs to the *P. conspicillatus* group. *Pristimantis adiastolus* was not included in our analysis, but its diagnostic external characters fit with the diagnosis of *P. conspicillatus* species. In addition, Padial *et al.* (2014) placed *P. adiastolus* in *P. conspicillatus* group based on molecular evidence. The diagnosis of the species *P. avicoporum* and *P. metabates* match the characteristics shared by *P. conspicillatus* species, however these two species are known by very few individuals, therefore we prefer to leave these species pending a group assignment. The same situation applies for *Pristimantis nebulosus*, Duellman & Lehr (2009) after studying the holotype concluded that this is a questionable species, the inadequate description and a poor condition of the holotype, make it difficult to associate this nominal taxon with any know population.

We could not access tissues and include them in our phylogeny of the following: *P. ixalus, P. carlossanchezi*, *P. epacrus*, *P. padrecarlosi* (Cordillera Oriental), *P. johannesdei* and *P. viridicans* (Cordillera Occidental), and *P. pedimontanus* (Cordillera de Merida) but their external morphology and cranial osteology differed well those of *P. conspicillatus* group, therefore they are excluded of the *P. conspicillatus* group. In summary, *P. conspicillatus* group includes twenty five species: *P. achatinus*, *P. adiastolus*, *P. bipunctatus, P. buccinator, P. dundeei, P. citriogaster, P. chiastonotus, P. condor, P. conspicillatus, P. koehleri, P. fenestratus, P. gaigei, P. gutturalis, P. lymani, P. malkini, P. paulodutrai, P.*

peruvianus, P. ramagii, P. samaipatae, P. skydmainos, P. terraeboliviaris, P, ventrigranulosus, P. vilarsi, P. vinhai, and P. zeuctotylus.

The potential number of unnamed species in the group on the basis of molecular data.

In addition to the currently twenty-five recognized nominal species, at least eight divergent lineage are identified in the results of phylogenetic analysis. In our topology is clear that *P. fenestratus* is the species with the most taxonomic problems, in part because of its wide range of distribution reported, Amazon Basin of eastern Peru, north eastern Bolivia, southeastern Ecuador and Colombia and Brazil, 100-1800 m elevation. The syntypes of *P. fenestratus* was described from Rio Mamoré (Rondonia, Brazil) and Borba (Amazonas, Brazil). Two different lineages identified as a *P. fenestratus* are from Ariquemes and Parecis (Rondonia. Brazil) nearby Rio Mamoré, one of these is close to *P. vilarsi* and *P. zeuctotylus* and the other one is closer to *P. ventrigranulosus*. Another different lineage identified as *P. fenestratus* from Pará (MT54, MT108, MUZUSP139440) and Maranhão (MT85) are more closely to *P. chiastonotus*. Finally, the populations of *P. fenestratus* from Peru and Bolivia (MNK6629-6631) are more closely related to *P. koehleri*, another linage. The major problem with *P. fenestratus* is that it lacks a good description and requires a redescription.

Our results suggest the existence of another species within nominal *P. gaigei*. The different populations of *P. gaigei* show genetic distance ranging between 3.3 % and 9.4 % in populations of Panamá and Magdalena River Valley, and divergences ranging between 2.02 % and 4.8 % among samples of the Magdalena River Valley in Colombia. We did not study carefully *P. gaigei*, but so far we have not found differences. Lynch (1980) showed a marked mimetic color pattern in specimens of Panamá, absent in the populations of in the river valleys of the Sinú, Cauca, and Magdalena in Colombia. In the same way, under the name of *P. achatinus* is more than one species. It is clear that *P. aff. achatinus* from Cordilleras Occidental and Central is a different lineage from *P. achatinus* from biogeographic Choco. However, many questions remain unresolved for *P. achatinus*. The type locality of *P. achatinus* is Cachabi, Esmeraldas, on the pacific coast of Ecuador. Therefore, we considered that the populations of Manabí-Ecuador (KU217809) and Buenaventura-Colombia (MT165) and Darien-Panama (AJC9573) are "nearby areas" from type locality. Genetic divergence ranged from 4% to 5% among populations of Ecuador and Colombia-Panama, suggest that they may be different species, but we still have no morphological evidence. Lynch & Myers (1983) found differences in coloration pattern and vocalization between populations of Pacific Ecuador and Panamá. *Pristimantis vilarsi* is paraphyletic because the populations from Mitu (Vaupes-Colombia) is sister to a clade composed of *P. zeuctotylus* and *P. vilarsi* populations from Colombia and Venezuela. Likewise, parsimony analysis reveals *P. malkini* from Colombia to be paraphyletic because is the taxon sister of clade composed of *P. malkini* from Ecuador and *P. citriogaster*. A direct examination of the vouchers and deep morphological studies are needed in order to understand the identity and limit of distributions of these widespread species.

The *Pristimantis* species removed from *P. conspicillatus* group distributed in Colombia.

Similarly to other terrarana molecular phylogenies (Hedges *et al*., 2008; Pinto-Sánchez *et al*., 2012; Padial *et al*., 2014) this study shows that *P. actites, P. caprifer, P. carranguerorum, P. fallax, P. lanthanites. P. medemi, P. megalops, P. savagei, P. thectopternus and P. w-nigrum* are not members of the *P. conspicillatus* group. With respect to *P. w-nigrum*, our result indicate that in Colombia under the name *P. w-nigrum* is more than one species associated with particular geographical regions: Cordillera Occidental and Cordillera Central. In populations of Ecuador, *P. w-nigrum* has deep divergences that are geographically significant, showing that *P. w-nigrum* is a species complex in Ecuador (Kieswetler & Schneider, 2013). *Pristimantis w-nigrum* has a wide distribution range from humid Pacific and Amazonian slopes of the Andes in Colombia and Ecuador. In Peru, it is known from montane forest localities in the northern part of the Cordillera Occidental. *Pristimantis fallax*, *P. lanthanites* and *P. thectopternus* are clustered with the "*P-w-nigrum*" of the Cordillera Central in Colombia. *Pristimantis lanthanites* occurs in the piedmont of the Cordillera Oriental of Colombia and in lowland Amazonian rainforest of Colombia, Ecuador and Peru. Pairwise distance between *P. lanthanites* from Colombia and Peru are high (5.1%), but absence of sufficient phenotypic information from parts of its geographic range prevent the suggestion of two different lineage between these two populations. *Pristimantis lanthanites* is closely related to *P. fallax* (distributed in the Cordillera Central, and considered by Padial *et al.* (2014) as an unassigned to species group), sharing conical supra-anal warts and color pattern on the throat (gular stripe). Although we do not have sequences of *P. epacrus*, we propose that this species is close to *P. lanthanites* and *P. fallax*, given the presence of supra-anal warts and color pattern on throat. We therefore transfer *P. fallax* to the *P. ridens* group, and *P. epacrus* is maintained in the *P. ridens* group on the basis of morphological evidence.

This study shows that *P. megalops* (which inhabits the highlands of the Sierra Nevada de Santa Marta) is cluster to *Tachiromantis douglasi*. Lynch (1996) reported the fusion of the 8th vertebrae and the sacrum in *P. delicatus* (which inhabits the highlands of the Sierra Nevada de Santa Marta) and *T. douglasi* (which inhabits the northern Cordillera Oriental of Colombia) In this study we found that other *Pristimantis* species which occur in the SNSM also have the same fusion (*P. carmelitae*, *P. cristinae*, *P. insignitus*, *P. megalops*, *P. ruthveni* and *P. sanctamartae).* Although we did not obtain sequences for *P. carmelitae* and *P. insignitus* (species assigned to *P. conspicillatus* group by Lynch & Duellman, 1997), we removed these two species for the *P. conspicillatus* group on the basis that these two species are probably nested with *Tachiromantis douglasi* and "*P. megalops",* under the assumption that fusion of the 8th vertebrae and the sacrum is a shared derived character. We propose that the *P. galdi* group must be dismantled, leaving *P. galdi* and *P. tribulosus* as the sole known species of *P. galdi* group. These species are very similar in bearing many conical tubercle along the body and in having green color, these also have cranial crests and bony tubercles along the lateral edge of frontoparietals.

Morphological characters

Two characters analysed in this study are significant for the systematics of the genus *Pristimantis*, the supra anal warts proposed by Lynch & Suarez (2000) and the size of the palatines proposed here. Although a large majority of characters is not informative in the historical context, some of them are useful in the characterization of the *P. conspicillatus* group and for some clades within the group. We corroborated that the relative lengths of the fingers I and II, skin of venter, texture of the skin: dorsolateral folds, tubercles on upper eyelid, and on heel, tympanum, and shank size are very useful in the taxonomy of *Pristimantis*.

Osteological characters as a directionally of the alary process, shape of the squamosal ventral ramus, length of the otic rami of the squamosal, length of the squamosal, are helpful to characterize clades in Brachycephaloidea. Furthermore, sexual secondary character as absence and vocal slits and presence of cranial crest are useful to diagnose clades in *Pristimantis*. Some of the external characters studied were modifications of characters defined by Lynch & Duellman (1997). Although knowledge of the systematics of the phylogeny terraranas has grown significantly in recent years, these species deserve a lot more attention in morphological studies in order to propose clear diagnostic characters and synapomorphies.

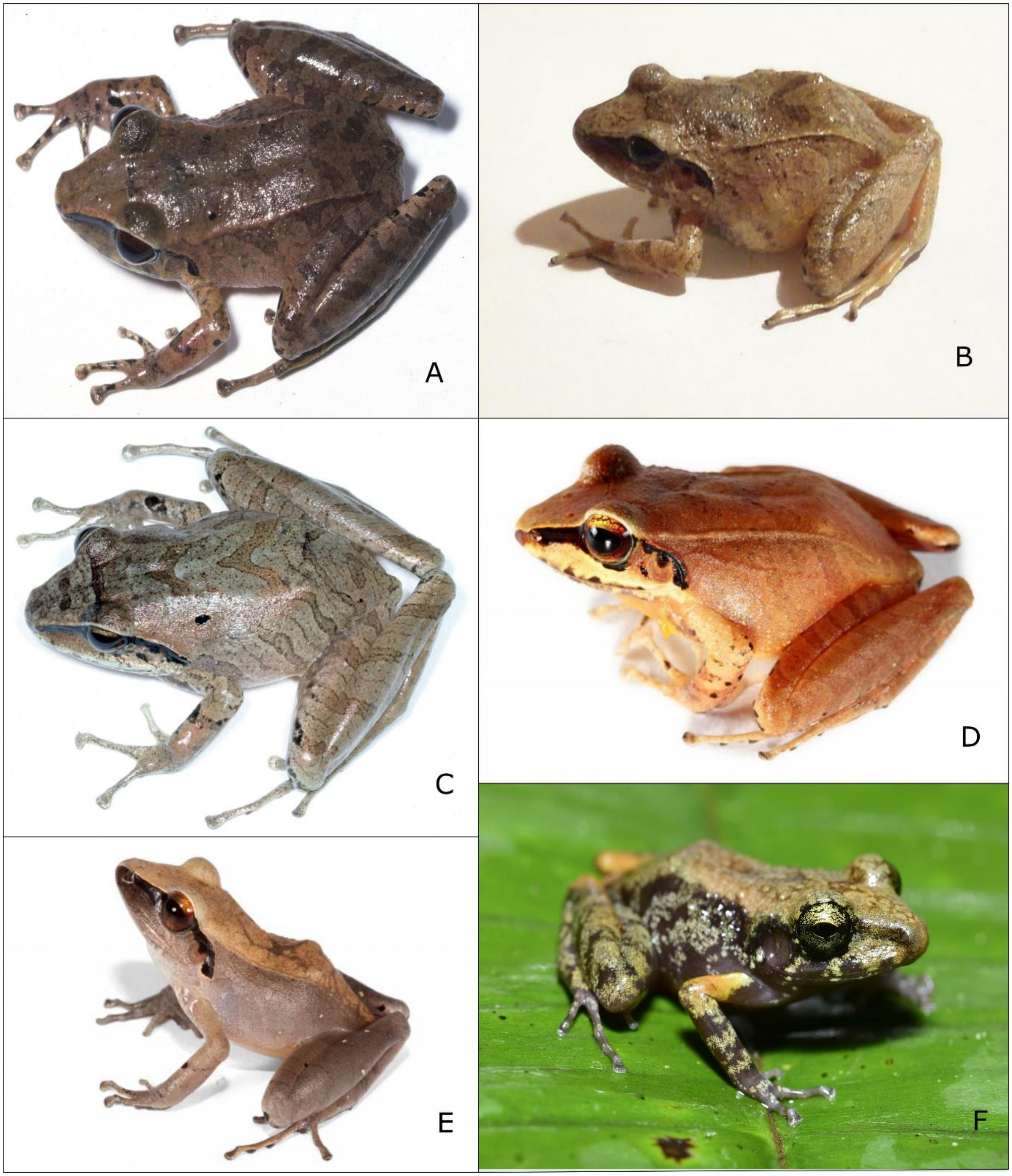

Figure 24. A) *Pristimantis adiastolus (*Photo: Hedges*)*. B) *Pristimantis aff. achatinus* (Photo: S. Arroyo). C) *Pristimantis bipunctatus* (Photo: Hedges). D) *Pristimantis condor* (Photo: S. Ron). E) *Pristimantis conspicillatus* (Photo: S. Ron). F. *Pristimantis gaigei* (Photo: S. Arroyo).

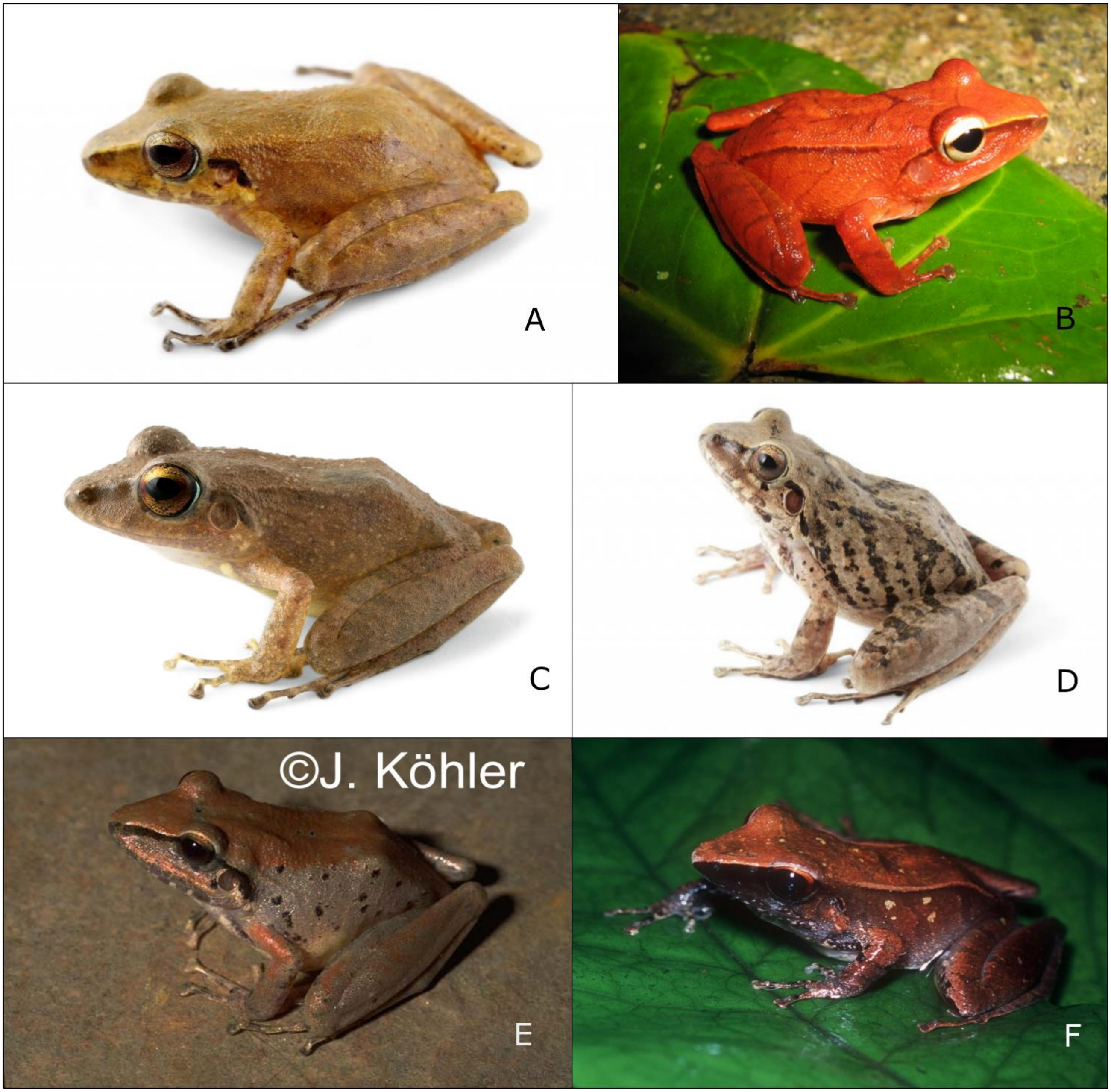

Figure 25. A) *Pristimantis citriogaster* (Photo: S. Ron). B) *Pristimantis achatinus* (Photo: J. Saria). C. *Pristimantis malkini* (Photo: S. Ron). D. *Pristimantis lymani* (Photo: S. Ron). E. *Pristimantis samaipatae*. F. *Pristimantis skydmainos*.

Chapter 3. Historical Biogeography of the P. conspicillatus group

Introduction

In the origin of the montane Andean flora and fauna two general hypotheses have been proposed. The most widely cited is that montane organisms have been largely derived from lowland relatives via long-distance dispersal (Monasterio & Vuilleumier 1986; van der Hammen & Cleef 1986) or by colonization as populations expanded their ranges upslope, became isolated due to climatically induced habitat change, and then differentiated (Brumfield & Edwards 2007). Another hypothesis is that ancestral populations were distributed across both lowland and pre-Andean landscapes, with tectonic uplift passively transporting some to increasingly higher elevations where they differentiated within a variety of montane habitats (Lynch, 1996). In summary, tropical montane fauna can evolve upward or downward across elevation gradients, through vicariance, horizontal speciation, and/or colonization from higher latitudes between mountain chains (Willmott *et al.*, 2001).

Similarity, several historical hypotheses have been proposed to explain the extraordinary species richness of the Amazon basin (Cheviron *et al.*, 2005; Wüster *et al.*, 2005), and these hypotheses generally invoke vicariance. The hypothesized causes of vicariant events include riverine barriers (Wallace, 1852), marine incursions (Nores, 1999; Webb, 1995), palaeoclimatic shifts resulting in forest refugia (Haffer, 1969, 1997), and climatic disturbance (Colinvaux, 1998). Parapatric speciation is also proposed to explain the diversification in the Amazon basin by divergent selection across ecological gradients (Endler, 1977).

Lynch & Duellman (1997) suggest that vicariance models provide the most parsimonious explanation for the pattern of distribution in the genus *Pristimantis.* The band hypothesis is highly related with niche conservatism pattern and predicts that within each montane system, highland taxa are more closely related to other highland taxa than to lowland taxa. Mendoza *et al.* (2005) identified the middle elevation band of the North-western Andes in Colombia and Ecuador as the most important region for origin and diversification of *Pristimantis*, and found a concordant pattern of diversification inside the middle elevation band between lowland and midland for some subgroups of *Pristimantis*. They identified the Northern Andes as the origin for *Pristimantis*; this pattern coincides with those previously identified by Pinto-Sánchez *et al.* (2012) in *Pristimantis* and for other amphibians and birds (Santos *et al.*, 2009; Bonaccorso & Guayasamin, 2013 and Castroviejo-Fisher *et al.*, 2014).

With a phylogenetic hypothesis for a monophyletic group containing a mixture of lowland and montane species, coupled with detailed geographic and elevational range data, it is possible to understand diversification of montane and Amazon fauna. Members of the *Pristimantis conspicillatus* group have a large and interesting distribution. These species occur from Costa Rica to Bolivia across the Andean Cordillera, the Amazon and Cerrado, the Guyana and in the Atlantic Forest; and have a range from the lowlands up to about 3000 m.a.s.l. Given that species of the *P. conspicillatus* group are distributed in the highlands and lowlands, their phylogenetic relationships can provide more evidence for our understanding of the diversification of the genus *Pristimantis.* Based on the hypothesis of phylogeny of *P. conspicillatus* group the questions in this chapter are the following: 1) Are lowland species of *P. conspicillatus* group more closely related to other lowland species or to highland species?, 2) What was the most probable ancestral distribution area for *P. conspicillatus* group?, 3) Does the radiation of the *P. conspicillatus* group follow a south to north pattern of speciation?, 4) Can the *P. conspicillatus* diversification be attributed to allopatric/vicariant scenario?

Materials and Methods

Historical biogeography is a discipline that studies the causal processes (historical and ecological) to reconstruct the pattern of distribution of organisms (Sanmartin, 2014). Cladistic biogeographical methods (also called 'pattern-based'; Sanmartin, 2014) were designed to find a general pattern of relationships among areas of endemism patterns without taking the underlying evolutionary processes into account (Ebach *et al.*, 2003; Parenti, 2006). The biogeographical processes like dispersal, extinction and vicariance were considered only *a posteriori* to explain cases of incongruence between individual phylogenies and general area cladogram. A new generation of methods for biogeographic inference was developed in the 1990s, Event-Based Biogeography (DIVA; Ronquist, 1997), represent an significant step forwards in that they use a deterministic cost-model in which each biogeographical process is assigned a fixed cost according to its probability or likelihood of occurrence (Ronquist, 2003). However, one criticism for these two methods (pattern and event based) is the use of principle of parsimony as the inference criterion in biogeographical analysis. Under this criterion, sources of priori evidence other than the tree topology and the species distribution, such as molecular estimates of the time of divergence between lineages (Donoghue & Moore, 2003), the fossil record or the connectivity of biogeographical areas on past (Sanmartin *et al.*, 2001) can only be incorporated indirectly into the biogeographical analysis (Sanmartin *et al.*, 2008).

Time in biogeography

By ignoring the time dimension in Biogeographical analysis, it is possible to fall into the phenomenon of biogeographical pseudocongruence. Geographic barriers are cyclical, and they may have been formed at different times, in which case it could not have been caused to the same vicariant process (Donoghue & Moore, 2003). Another reason to include time in biogeographic analysis comes from the disciplines of palaeontology, geology and geography. Plate tectonics move over the mantle transporting with them the continental crust and oceanic and therefore the position of these plate tectonic change over time. This means that areas and their biotas split up, fuse and split up again in repeated cycles of biogeographical process as geographical barriers appear and disappear over time (Donoghue & Moore, 2003). Therefore, taking into account the temporal dimension (time) may help with problems associated with biogeographical pseudocongruence and reticulate biogeography.

Parametric models

Other approach has been developed that is not constrained by the inherent biases of parsimony criterion (model based or parametric models; Ree & Sanmartin, 2009). Parametric methods model the biogeography of lineage (the change in geographical range from ancestor to descendant) as a stochastic process with discrete states that evolve along the branches of the phylogeny according to a probabilistic Markov chain model (Ree & Sanmartin, 2009). One of the advantages of parametric method is that they allowed the integration into biogeographical inference of estimates of evolutionary divergence between lineages (length branches). Moreover, parametric method can account for the uncertainty in ancestral range reconstruction because all possible biogeographical scenarios are evaluated by estimating the relative probabilities of ancestral areas (Bureki, 2014). Dispersal-Extinction-Cladogenesis (DEC) is a parametric method that allows estimating the rate of biogeographic change, expressed as dispersal and extinction parameters and the ancestral geographical ranges at each node in a phylogeny using maximum likelihood statistical inference (Ree &Smith, 2008).

Ancestral area reconstruction of *Pristimantis conspicillatus* group

The distribution range of the Terrarana was divided into eleven areas. We followed Duellman (1999) and Castroviejo-Fischer *et al.* (2014) in the delimitations of areas for amphibian in the Neotropics. Some of these areas were modified to better fit the geographic distribution and pattern of endemism within terrarana with emphasis in *P. conspicillatus* group (Figure 23; see Appendix 6 for distribution of each species). (A) **The Northern Andes**: (12° N–5° S). 2000 km long, have a NNE–SSW orientation, and extends from easternmost Venezuela to northernmost Peru around a mega shear zone called the Amotope Cross at *c.* 2° S near the Gulf of Guayaquil (Graham, 2009). It includes eastern and western cordillera in Ecuador, the three cordilleras in Colombia, Nudo de Pastos, inter-Andean basins (Magadalena and Cauca), Serranía del Perijá and Cordillera de Mérida. Most of the diversity of the genera *Hypodactylus, Pristimantis* and *Lynchius* are distributed in this area. *Pristimantis aff. achatinus*, *P. condor* and *P. lymani* are the species of the *P. conspicillatus* group found in this area. (B) **Chocó**: Pacific coast of northern Ecuador, Colombia, Panama and Costa Rica. Most of the diversity of the genus *Diasporus* occurs in this area, the genera: *Craugastor*, *Pristimantis* and *Strabomantis* also occur in Chocó. *Pristimantis achatinus* and *P. gaigei* are the species of the *P. conspicillatus* group found in this area. (C) **Central America**: From the northern rain forests of Mexico to the Isthmus of Panama. Most of the diversity of the genus *Craugastor* is distributed in Central America. None of the *P. conspicillatus* species group is distributed in this area. (D) **Antilles**: The Greater (Cuba, Jamaica, Puerto Rico, Hispaniola and Cayman Island) and Lesser Antilles (Leewars Islands, the south-easterly Windward Island and the Leeward Antilles). Most of the diversity of the genus *Eleutherodactylus* is distributed in the Antilles. None of the *P. conspicillatus* species group is distributed in this area. (E) **Atlantic Forest**: Stretching along the coast of Brazil from the states of Rio Grande do Norte to Rio Grande do Sul. It also extends inland into eastern Paraguay and the Misiones province of northeast Argentina, and includes several islands off the Brazilian coast, such as the archipelago of Fernando de Noronha. The Atlantic Forest can be considered an island, because it is isolated from other large blocks of Soith America forest (Amazonian and Andean forest) by a corridor of open to semiopen formations, comprising Caatinga, Cerrado and Chaco (Ab'Sáber, 1977). In terrarana the genus *Brachycephalus* and *Ischnocnema* are endemic to the Atlantic Forest. *Pristimantis paulodutrai*, *P. ramagii* and *P. vinhai* are the species of the *P. conspicillatus* group found in the Atlantic Forest. (F) **The Central Andes**: From northern Peru to Bolivia and northern Chile. Separated from the northern Andes by the Huancabamba Depression (Jaillard *et al.*, 2000) approximately at the same latitude than the Amotope Cross. Most of the diversity of the genus *Psychrophrynella* occurs in this area, the genera: *Craugastor*, *Oreobates*, *Phrynopus*, *Pristimantis* and *Yunganastes* also occur in the Central Andes. *P. koehleri*, *P. lymani* and *P. samaipatae* are the species of the *P. conspicillatus* group that occur in the Central Andes. (G) **Guyana**: Lowlands and highlands corresponding to the Guiana Shield in northeast South America (Gibbs & Barron, 1993). This area includes the rain forests from the Orinoco to the Amazon River and east of the Andes. *Adelophryne*, *Ceuthomantis* and *Pristimantis* are the genera of Terrarana distributed in Guiana Shield. *Pristimantis chiastonotus*, *P. gutturalis*, *P. vilarsi* and *P. zeutoctylus* are the species of the *P. conspicillatus* group found in this area. (H) **Amazon**: Lowland rain forests (< 500 m a.s.l.) east of the Andes in Bolivia, Brazil, Colombia, Ecuador, and Peru but excluding the Guiana Shield. Terrarana is distributed in the Amazon except for the genera: *Atopophrynus, Barycholos, Craugastor, Diasporus, Eleutherodactylus, Euparkerelella, Geobatrachus, Haddadus, Holoaden, Phrynopus* and *Niceforonia.* With the new circumscription of the *P. conspicillatus* group, most of the diversity of this group occur in Amazonia, *P. buccinator*, *P. citriogaster*, *P. conspicillatus*, *P. fenestratus*, *P. gutturalis*, *P. lanthanites*, *P. malkini*, *P. peruvianus*, *P. skydmainos* and *P. vilarsi* inhabits this area. (I) **Sierra Nevada de Santa Marta (SNSM)**: A relatively small mountain chain in the north of Colombia, within three departments of the Colombian Caribbean, it is isolated from other rain forests by dry valleys. This area, comprises multiple ecosystems, including dry and wet tropical forests, sub-Andean and Andean forests, moors and zones with perpetual snow cover (PNNC 2015; ProSierra 2015). With around 17 species of amphibians, 12 of reptiles, 14 of birds and one mammal, all of them endemic to the area (UAESPNN 2005), it is considered one of the greatest centers of endemism in the country and one of the irreplaceable protected areas of the world (Le Saout *et al.* 2013; Lynch *et al.* 1997). *Geobatrachus* and *Pristimantis* are the genera of Terrarana distributed in SNSM. None of the *P. conspicillatus* species group is distributed in this area. (J) **Cordillera de la Costa**: This region stretches from east to west in a narrow band alongside the Caribbean Sea. The Cordillera principally consists of two mountain chains separated by high valleys, of which the best known is the Caracas valley. *Pristimantis terraebolivaris* is the species of *P. conspicillatus* group found in this area, and it is endemic. (K) **Caatinga**: This area is distributed throughout the northeast region of Brazil (except for the State of Maranhão) and the northern part of the State of Minas Gerais, and represents 11% of the Brazilian territory. The Caatinga biome is characterized by semiarid to arid climates (Santos & Tabarelli, 2003) with long dry seasons, irregular rainfall (concentrated in the summer), average annual precipitation of 400 to 600 mm (Ab'Sáber, 1977). *Haddadus* is the genus of Terrarana found in this region. (L) **Cerrado**: The Brazilian savanna vegetation is called Cerrado. The Cerrado region extends from the margin of the Amazonian forest to outlying areas in the southern states of São Paulo and Paraná, occupying more than 20° of latitude and an altitudinal range from sea-level to 1800m. A few species of *Oreobates* and *Pristimantis* occur in Cerrado, and the only two species of *Pristimantis* that inhabits the Cerrado belong to the *P. conspicillatus* group: *P. dundeei*, *P. ventrigranulosus and* undescribed species.

Biogeographical inference

We inferred the most probable ancestral distributions for the internal nodes within the phylogenetic tree using Dispersal–Extinction–Cladogenesis (DEC) model (Ree et al., 2005; Ree & Smith, 2008), implemented in RASP, and using the BEAST calibred phylogeny (Chapter 2 for details). We applied stratified model that incorporates a dispersal multiplicative matrix between areas at different periods in time. According to the most relevant paleogeographyc events in the area of distribution of *P. conspicillatus* group, we differentiated tree time periods (see Appendix 7 for the multiplicative matrix used). 1) From Eocene to Middle Miocene (49-15 Ma). During this time, Choco and Amazonia regions were connected and ca. at 23 Ma Lake Pebas was stablished. During this period was the origin of the genus Pristimantis and the North-western Andes was probably incipient (at less the eastern Colombian cordillera); in addition, Atlantic Forest and Amazonia were connected (ranging from 5.6 to 23 Ma) (Batalha-Filho et al., 2012). 2). Middle to late Miocene (15-5 Ma). During the Middle Miocene was the time of closure of the Central American Seaway (Montes el al., 2015); moreover, this period coincide with the disappearance of Lake Pebas. 3) From Pliocene (5 Ma) to present. During this period Central and North-western Andes were connected, and the North Andes accelerate his elevation. Furthermore, for connections between areas we generate the following values: 1.0 for pair of areas with direct connection; 05 for pair of areas separated by one region and for regions which are not directly connected but now have the same species living in both regions; and 0.1 for pair of areas with no direct connection, and with no evidence of sharing current Terrarana species.

Altitudinal reconstruction in Terarrana

We follow Lynch (1999) to determine the elevation intervals. In this sense, five categories are recognized according to the upper and lower limits of distribution of *Pristimantis* species in Colombia. A= species below 1000 m; B= species between 1000 and 1800 m; C= species between 1800 and 2700 m, D= 2700 and 3200 m, and E= species higher than 3200 m. Data for elevation ranges were obtained from Amphibian species of the world (Frost, 2015), Global Amphibian Assessment, Global Biodiversity Information Facility (GBIF), and publications of records and range extensions (Bernal & Lynch, 2008; Duellman & Lehr, 2009). Ancestral elevation was reconstructed using parsimony reconstruction with Mesquite 3.03 (Maddison & Maddison, 2015). In order to avoid bias by incomplete and not-random taxonomic sampling; for the analysis of altitudinal changes in Terarrana we select four clades, including *P. conspicillatus*. In this clades, we count the number of changes between altitudinal ranges, including the variation because acctran/deltran alternative reconstructions.

Andean *Pristimantis* clades selected for altitudinal reconstruction

According to the new circumscription of the *P. conspicillatus* group (chapter 2), most of the species are distributed in the Amazon and a few in the Andes. However, these Andean species are useful to test the hypothesis of Andean diversification. We take some Andean clades that have a good taxonomic sampling in order to understand the diversification in Andean *Pristimantis* species. The first clade to be analysed was selected because it has a good taxonomic sampling, and because it includes species that were allocated in the *P. conspicillatus* group, and now are part of the *P. ridens* group *sensu* Padial *et al.* (2014). The second group correspond to *P. devillei* group and the last clade is the *P. danae* group, which is composed mostly of species from the Central Andes and contiguous Amazonia

Results

Diversification of *P. conspicillatus* group

The genus *Pristimantis* diverged from other Terrarana species in the Eocene 37.01 Ma (with 95% credibility interval, CI, of 28.49–45.47 Ma) and began radiating 32 Ma (CI: 24.91–39.55 Ma) (see Appendix 8 for DEC reconstruction in the whole phylogeny). Meanwhile, *Pristimantis conspicillatus* group began radiating at the Oligocene (24.26 Ma, CI: 18.53–30.22 Ma). The DEC analyses recovered Amazonian + North Andean as the most likely ancestral area of *P. conspicillatus* group. This event was followed by several events of colonization to other habitats. Four principal clades can be recognized, (Figure 25) 1) mostly species from Guyana and Amazonia, 2) mostly species from Amazonia and Guyana, 3) Andes and Amazonia, and 4) Cerrado and Amazonia.

Clades 1 and 2 are sister to a clade composed of *P. terraboliviaris* from the Cordillera de la Costa of Venezuela and *P. gutturalis* from Guyana, and the ancestral area of this whole clade is ambiguous between Amazonia, Guyana or Amazonia + Guyana. The divergence of these two species was probably through vicariance and occurred during the Middle Miocene (ca. 14 Ma). In the predominantly Guyana clade 1, several putative dispersions and a vicariance are reconstructed as the process responsible to the origin of the six or seven species. On the other side, the clade 2 is almost Atlantic forest restricted, but with a largely disjunction with the sister species *P. gaigei*, distributed in the Chocó and Middle Magdalena regions. The ancestral areas of this clade are Amazonia and the Atlantic Forest about 20 Ma.

The third clade is mainly composed of Amazonian species, with ancestral area in the Amazonian + North Andean regions. Subsequently, one clade was restricted to Amazonia, and the other one remained in the Andean region, probably colonizing the Andes and Chocó in two separate events.

The clade 4 includes species from Amazonia, the Cerrado, the Central Andes and one from Guyana (*P. chiastonotus*). The ancestral area is ambiguous between Amazonia and Amazonia + Cerrado. Then, two clades with clear geographical affinities are defined, the first one reaching the Central Andes, and the second one in lowlands of Amazonia, the Cerrado and Guyana. Three events of vicariance are recognized: the first one between Amazonia and Central Andes (2 Ma), the second between the Cerrado undescribed species and "*P. fenestratus*" from Amazonian (about 7 Ma) and the last one between Amazonian species and *P. chiastonotus* from Guyana (about 10 Ma).

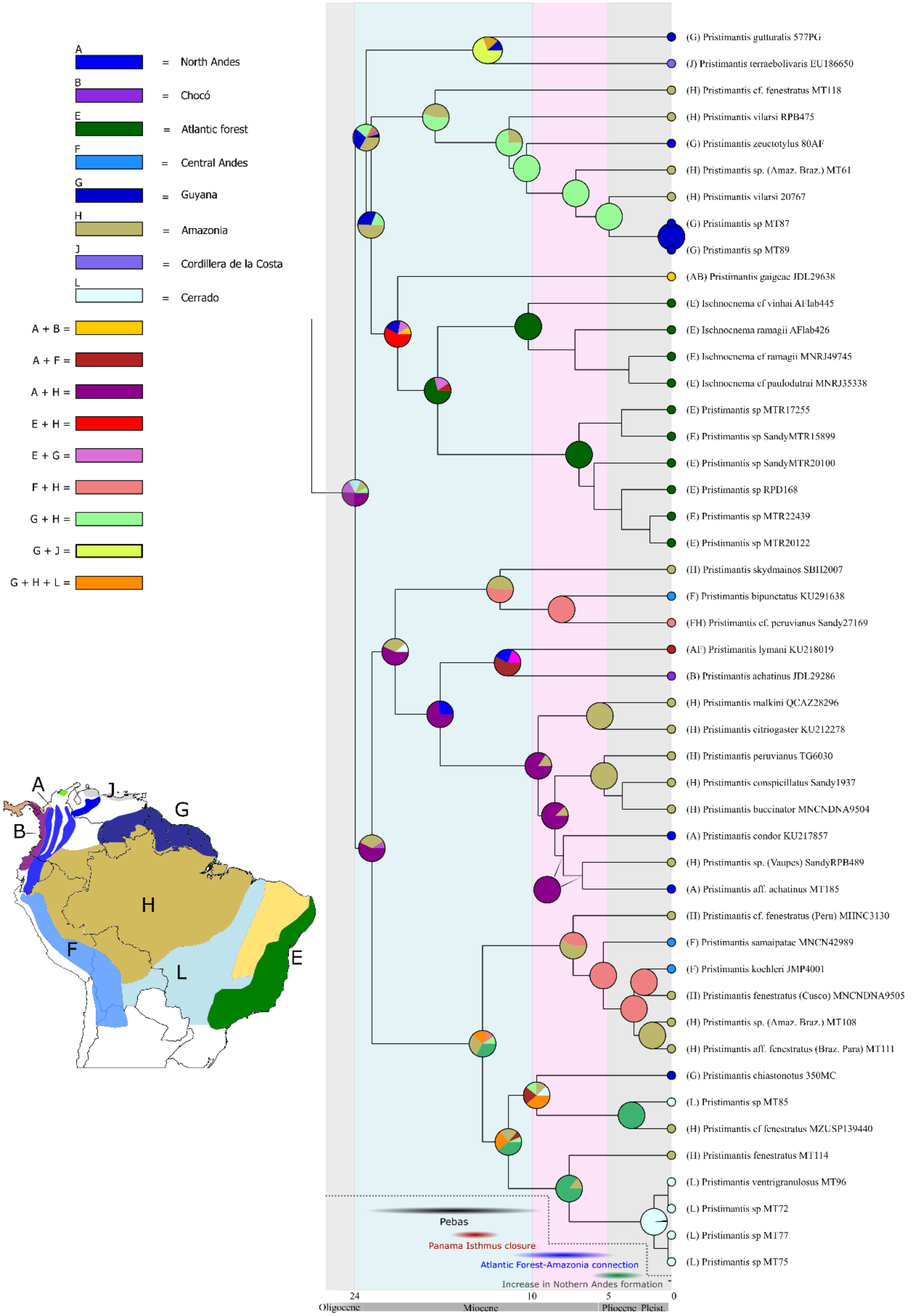

Figure 26. Biogeographical reconstruction of the *Pristimantis conspicillatus* group using BEAST calibred tree and DEC model of reconstruction of ancestral areas. On the lower

part the most important geological events that influenced the diversification of this group. On the left the color code and map with the regions considered for the DEC analysis.

Altitudinal changes in Terarrana

The origin of Terarrana was reconstructed in the lowlands and the origin of *Pristimantis* is ambiguous. The origin of *P. conspicillatus* group is also in the lowlands (figure 27), which is consistent with the fact that the Amazonia Amazonia + North Andes was the most likely ancestral area of *P. conspicillatus* group. Our analysis shows that there have been more altitudinal changes from lower to higher altitudinal ranges (Figure 26). The higher number of changes (ca. 25 depending on acctran/deltran optimization) was observed from the lowlands (A) to the sub-Andean forest (B). Most of the changes occurred between contiguous bands, except for several changes between A and C, and vice versa. The central bands have a symmetrical number of changes with lower and higher bands, with the exception of D, which has a few more changes to C than to E (figure 28). Finally, the colonization of high mountains have occurred independently for different clades.

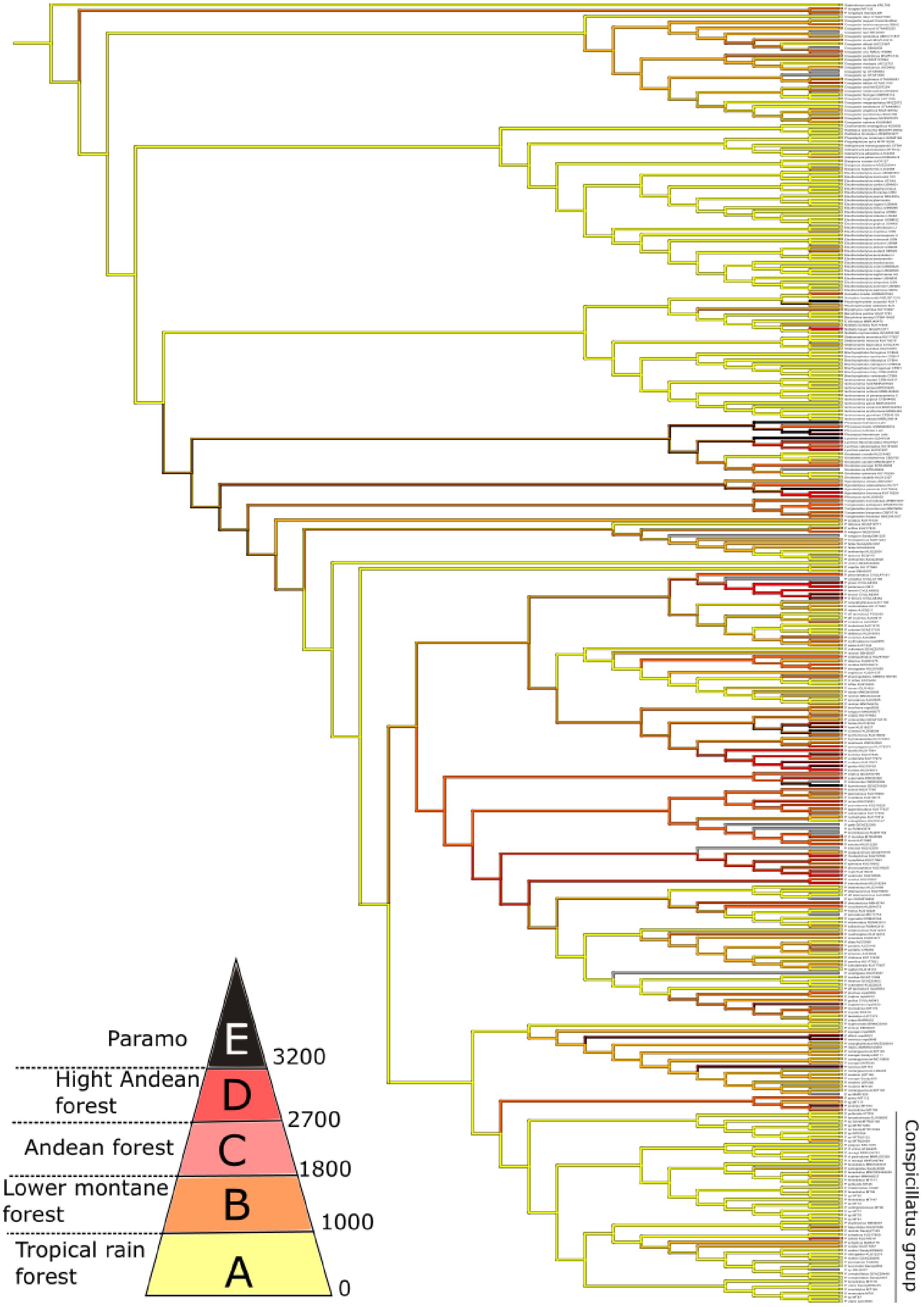

Figure 27. Altitudinal changes optimized on the MP phylogeny. Colours in the braches correspond with the color in the triangular representation of altitudinal ranges. The separation of altitudinal ranges fallow Lynch (2000).

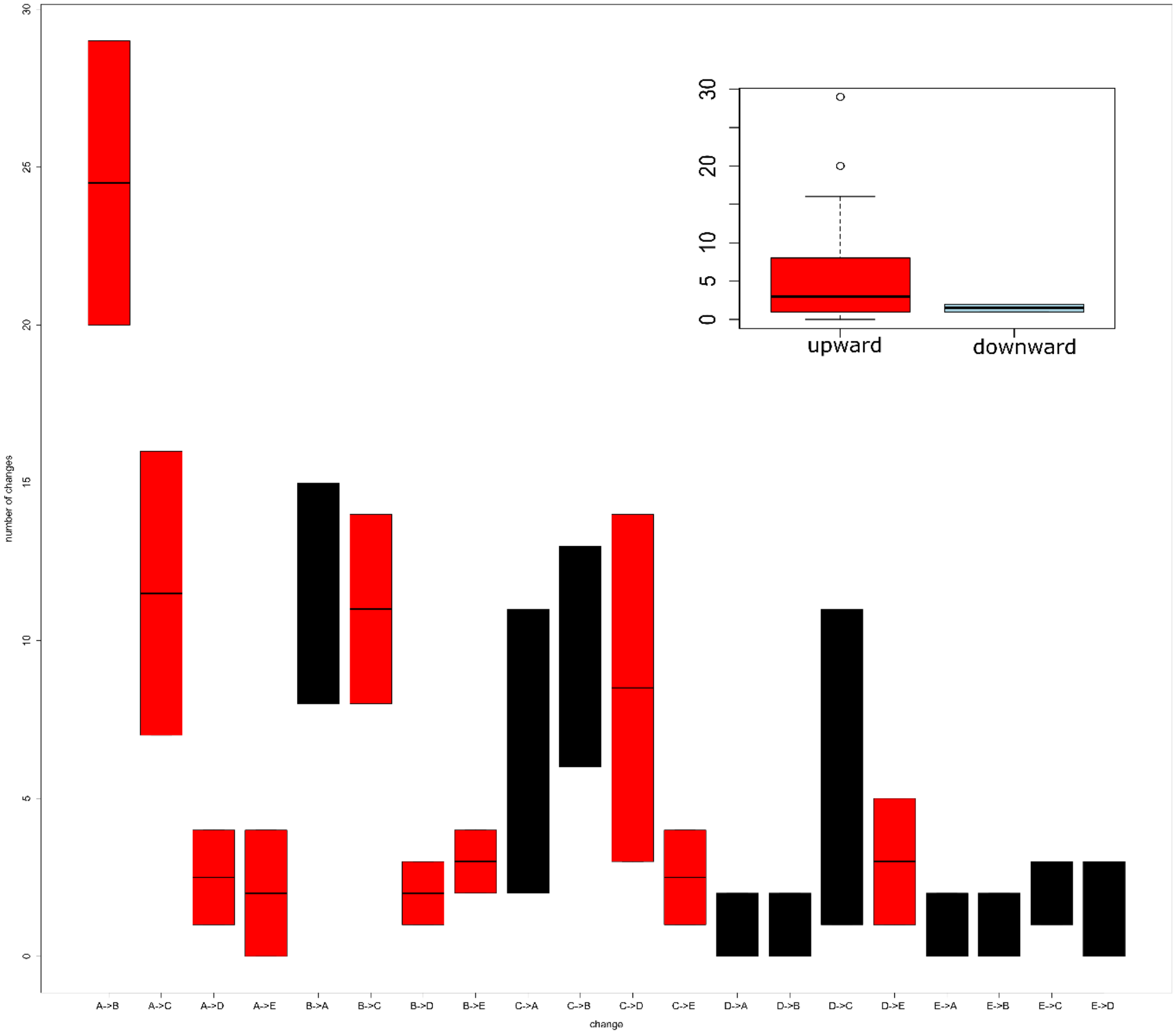

Figure 28. Frequencies of altitudinal changes between ranges, mapped on the phylogeny of Terarrana. The boxplot on the right shows a comparison of the quantity of changes upward vs. downward.

Discussion

Our results regarding the general directionality of diversification in the Andes in the genus *Pristimantis* are based on other studies in this genus, where a directionality of diversification from the north to the south Andes has been observed (Pinto-Sánchez *et al.*, 2012). This result is also observed in dendrobatids (Santos *et al.*, 2009) and centrolenid frogs (Castroviejo-Fischer *et al.*, 2014). However, in other taxa such as lizards, a pattern of cladogenesis following the rise of the Andes (Castroviejo-Fisher *et al.*, 2014). Consequently, the attempt to connect the geographical pattern of diversification in the Andes with the direction of the orogeny seems too simplistic. Some variables that may change the presumed pattern are: 1) a logical dependence of the region where the taxa originated, 2) if the principal events of diversification occurred before the Andean mountains were stabilized, then we would not expect a null hypothesis in the reconstruction of ancestral areas, such as older nodes in the Central Andes and, 3) even when, in general, the geological evidence shows that the orogeny of the Central Andes precedes the orogeny in the North Andes, the knowledge about the timing of orogeny in the Central and Western Colombian Cordilleras is comparatively poor (Gregory-Wodzicki, 2000), and some northern massifs may be very old. For example it is known that the Colombian Central Cordillera can be traced back to the late Cretaceous (Parra *et al.*, 2009).

The North Andes is the ancestral area of *Pristimantis*, which is consistent with other studies (Pinto-Sánchez *et al.*, 2012; Mendoza *et al.*, 2015). However, there are more changes between lower to higher altitudinal bands, which agree with Sedano & Burns (2010) for birds this might seem contradictory at first sight, but it can be explained because even when in many cases the ancestral area on internal nodes is the North Andes region, posteriorly the lowlands were colonized, and several more recent cases of “return” to higher zones produce the pattern of more rises. One interesting observation occurred in the band D (high Andean forest), where there are more changes to the Andean Forest (C) than to the paramos (E). This was noticed by Lynch (1999), who proposed that probably in *Pristimantis* species it is harder to adapt to the páramo compared to the lower bands. The fact that only in this band is this effect supports the idea that the opportunity of adaptation under the idea of glaciation signal.

Diversification in the P. conspicillatus group: The effect of the Pebas System

Even when remain some debate respect to the magnitude of the Pebas system (Wesselingh *et al.*, 2002; Latrubesse *et al.*, 2010), there is consensus that western Amazonia was dominated by aquatic settings between ca. 23 and 10 Ma (Hoorn et al., 2010). The effect of this system as a barrier for the Amazonian biota have been documented in terrestrial fauna for Dendrobates (Clough & Summers, 2000), Lachesis (Zamudio & Green, 1997) and lowland Mangoes (hummingbirds) (Bleiweiss, 1998). According to Wesselingh & Salo (2006) the Pebas system was determining in the diversification of Amazonian biota, fist acting as a barrier and after his demise by provide new and heterogeneous areas to colonization.

The radiation of *P. conspicillatus* group began ca. 24.2 Ma, near to the onset of the Pebas system, and our result indicate that his biogeographical history has since been closely linked to this marine incursion. A first pattern that can be explained by the existence of the Pebas system is the disjunction in the clade 2, between Atlantic Forest and Chocó-Middle Magdalena regions. Taking into account that there are few possibilities of long distance dispersal in amphibians, we postulate a plausible event of vicariance. The aquatic environments of the “Pebas” system could have produced extinctions in the central Amazonia and leaving only the species inhabiting at the Atlantic Forest (Eastern extreme) and the western Andean-Amazonian part. On the other hand, the connection between Amazonia and the Atlantic Forest has been dated between the Pliocene and late Miocene times (Patel *et al.*, 2011; Lutz *et al.*, 2013). And before the Late Miocene the Colombian Eastern Cordillera did not yet represent an important barrier between the Western Amazon and the Magdalena Valley (Hoorn *et al.*, 1995; Gregory-Wodzicki, 2000; Cediel & Shaw, 2003; Weir & Price, 2011; Ochoa *et al.*, 2012;). Given these connections, it is reasonable that between the late Miocene and Pliocene, the ancestor of this clade was separated by a long distance. Additionally, with reserve we think that the bigger genetic structure of the Middle Magdalena population of *P. gaigei* suggest that this was his original distribution, with posterior dispersion to the Chocó and Central America. However, this history have to be tested by phylogeographic methods.

During the existence of the Pebas system most of the ancestral areas reconstructed are out of the Amazonia. However, near of 10 Ma appears exclusively Amazonian species as *P. skydmanos*, and posteriorly ca. 6 Ma appears species in different clades as *P. malkini, P. peruvianus* and *Pristimantis cf. fenestratus (Peru_MHNC3130*). This is coincident with the regression of the Pebas system, and agree with the idea that the Western and central Amazonia was populated fundamentally by species derived from contiguous regions, as the Andes and the Guyana.

The species from the Cerrado

We hypothesize that the main process that explains the presence of *P. conspicillatus* group in Cerrado is ecological differentiation due to the emergence of the savannas of the Cerrado that drive parapatric speciation, rather than vicariance. The split between Cerrado and Amazonia occur in Middle Miocene, when most of the geophysical activity of the Cerrado took place (Uba *et al.*, 2006; Mulch *et al.*, 2010). Guarnizo *et al* (2015) also observed that some taxa from Amazonian are closely related to Cerrado and the split between these taxa are also during Late Miocene (6.4 Ma). Moreover, our estimated coincide with the young age of the Cerrado (Simon *et al.*, 2009).

The Atlantic Forest Clade

The Atlantic Forest is one of the best-defined biogeographical regions in South America and can be considered an island. The Atlantic Forest is isolated from the Amazonian and Andean forest by a corridor of open to semiopen formations consisting of Caatinga, Cerrado and Chaco (Ab' Sáber, 1977). We found two different clades in the Atlantic Forest, the first one is composed of *P. vinhai*, *P. paulodutrai* and *P. ramagii*, species distributed from Bahia to Alagoas and Paraiba. The second clade is composed of at least five undescribed species and is distributed from Bahia to Minas Gerais. Fouquet *et al.* (2012) found two different clades limited geographically between northern Atlantic Forest (from Ceará to Bahia), and southern Atlantic Forest (from Bahia to Minas Gerais) in the genus *Adelophryne.*

Furthermore, we estimate that northern Atlantic Forest clade diverged from southern Atlantic Forest about 17 Ma. In comparing this data with Fouquet *et al.* (2012), one can see that the patterns and timing of diversification are quite similar, they found northern Atlantic Forest/southern Atlantic Forest splits for *Adelophryne* (16 Ma). Similarly, the north vs. south Atlantic forest pattern observed within *Pristimantis conspicillatus* group and *Adelophryne* is concordant with several studies of vicariant forms whose limits are more or less coincident with the Rio Doce valley (northern Espírito Santo state; Pinto-da-Rocha *et al.*, 2005; Carnaval *et al.*, 2009**).**

Rivers and river valleys are frequently associated with genetic breaks in the AF, but their roles as primary barriers are questionable because their ages often do not match estimated divergence times (Thomé *et al.*, 2014). For instance, the Doce River lies over a complex area that shows geological changes as recent as 9000 years (Mello *et al.* 1999). Other barriers are even less evident, for instances, a disruption in climate is caused by the synergic effect of a cold oceanic upwelling north of the Rio de Janeiro (Araujo, 1997), and the increased distance of mountain ranges and the coast in the Atlantic

Forest slightly north of the Doce River (Thomé *et al.*, 2014). These factors affect the climate by decreasing rainfall and increasing seasonality, which correlates with several plant taxa that are restricted to either one of these regions, generating a strong floristic differentiation between northern and southern Atlantic Forest (Oliveira-Filho & Fontes, 2000). Because reproductive periods in anurans are strongly influenced by environmental variables, this climate disruption might constitute a significant cryptic barrier between northern and southern Atlantic Forest (Thomé *et al.*, 2014)

The Amotape-Huancabamba region

Pristimantis achatinus is a trans-Andean species, sister to *P. lymani* from the limit between the Central and the Northern Andes, this may document the transition between the Amazon and Chocó regions via the Amotape Huancabamba region. The biogeographical importance of this region has been recognized (Weigend, 2004), and our results support the idea that the large diversification in this region is not only due to the overlap between the North and Central Andes, and to the *in situ* climatic and geological history, but also due to the transition between *cis* and *trans* Andean taxa. The sister pair *P. malkini* + *P. citriogaster* occurs in an ecological gradient, with *P. malkini* in the Amazonian lowlands and *P. citriogaster* in the foothills of Peru and Ecuador, and in this case ecological speciation is supported. Meanwhile, the sister pair *P. buccinator* and *P. conspicillatus* have clearly allopatric distribution, with *P. buccinator* to the south of the Marañón-Amazonas River in Peru and *P. conspicillatus* to the north of this river, in similar habitats, that agree with the areas of endemism in Inambari and Napo (Lutz *et al.*, 2013)

Changes in altitudinal ranges thought the Phylogeny of Terarrana

Changes in the temperature along the altitudinal gradient of the Andean tropics represent an important limiting factor to the amphibians (Bernal & Lynch, 2013). Araujo *et al.* (2013) shows that evolution of tolerance to cold is more frequent than tolerance to warm. Based on this behaviour of the physiological tolerance to the temperature changes, we can expect that parapatric speciation along altitudinal gradient upward is most frequent than downward, as we in fact found for Terarrana. However, our result also depict a difference in this general pattern at the highest altitudinal ranges, where the changes upward are less frequents. We consider that this obey to two factors: 1) the top of the mountains are in general smaller, and 2) the high mountains zones of the Andes are comparatively much more recent that the middle mountains (van der Hammen & Gonzalez, 1953; Gregory-Wodzicki, 2000).

Andean *Pristimantis* clades selected for altitudinal reconstruction

The *P. ridens* group, the distribution of the species is allopatric and shows latitudinal and altitudinal replacement. *Pristimantis w_nigrum* complex (including *P. actites*) is distributed in the middle elevation of Ecuador, a new species usually incorrectly identified as *P. w_nigrum* that occurs in the highlands of the Cordillera Central in Colombia (CCC), *P. thectopternus* distributed in the middle elevation of the Cordillera Occidental de Colombia (CWC) and the western flank of the CCC, *P. aff. fallax* endemic to the Serrania de los Yariguies, in the Cordillera Oriental de Colombia (CEC), and *P. lanthanites* distributed in lowland Amazonia and lower humid montane forest from Peru to Colombia. Based on the altitudinal optimization and the ancestral areas reconstruction, the origin of this clade was probably the humid rainforest of the CCC. The *P. w_nigrum* complex, replaces latitudinally the rest of the species in the Ecuadorian mountains, meanwhile in the CCC one species reaches the highland (not described), and one clade reaches probably the proto Colombian Eastern Cordillera, and subsequently is split into *P. lanthanites*, and *P. aff. fallax*, from the western basin of the CEC. Therefore, for this clade the model of speciation in Andean anurans proposed by Lynch

(1986), agrees with our results, even when the relationships among the species have been changed.

The second group (Figure 27) is composed of species of the *P. devillei* group (Padial *et al.*, 2014), and formerly included in three different groups by Lynch & Duellman (1997). According to Lynch & Duellman (1997), in each of these groups the distribution of the species is usually allopatric. However, as shown in the topology the distribution of these species have no geographical pattern, but the species are mostly sympatric. Even when the sister species (*P. gentryi*, *P. truebae*) and (*P. buckleyi*, *P. vertebralis*), are sympatric, they have a partial altitudinal differentiation, with *P. gentryi* and *P. buckleyi*, inhabiting higher elevations than the respective sister species. In this case, the model of allopatric speciation is not supported, or the geographical signal may have been erased, however the altitudinal differences suggest parapatric speciation under the altitudinal gradient, with secondary contact.

A third clade is the *P. danae* group, which is composed mostly of species from the Central Andes and contiguous Amazonia, has narrow distributions in eight of the eleven species. This group probably originated in the lower montane forest of the Central Andes. The nominal *P. danae* from Bolivia is placed in our phylogeny as sister to *P. reichlei* and paraphyletic with respect to *P. danae* from Peru (where it is the type locality), that suggest the existence of two species of allopatric distribution with latitudinal replacement. According to Padial & de la Riva (2009), the speciation of the sister species *P. reichlei* and *P. danae* was probably associated with humid refuges, but the parapatric distribution of these species in one ecological gradient from the lowland to the montane forest may equally support one event of ecological speciation. The sister pair *P. toffae* and *P. rhabdolaemus*, have altitudinal replacement, with *P. toffae* in the Amazonian lowlands between 200 and 900 m, and *P. rhabdalaemus* in the lower montane forest, between 1000 to 2450 m

(Frost, 2015). The distribution of P. *pharangobates* is partially overlapped with *P. rhabdalaemus*, occurring in similar altitudinal ranges, however, *P. pharangobates* reaches southern areas in Bolivia. The sister species to the previous clade is *P. sagittulus*, that is endemic to the Cordillera Yanachaga in Peru, farther north than all the other montane species cited.

The last subclade to analyse in the *P. danae* group is composed of four species with narrow distributions. *Pristimantis stictiogastes* is allopatric with respect to its sister species, *P. ornatus*, and these two are separated by the Oxapampa valley. The two remaining species in this clade, *P. albertus* and *P. aniptopalmatus* are endemic to the Yanachaga Cordillera, but occur in different altitudinal ranges, with the most external species (*P. aniptopalmatus*) in higher altitudes (2300-2900 m). The orographic associate environmental gradients in the Cordillera Yanachagua were studied by Catchpole & Hons (2012), who found strong effect of the fog presence on the microclimate. Probably the altitudinal replacement in the very species-rich Cordillera Yanachagua, and others Andean-Amazonian massifs respond to these environmental gradients.

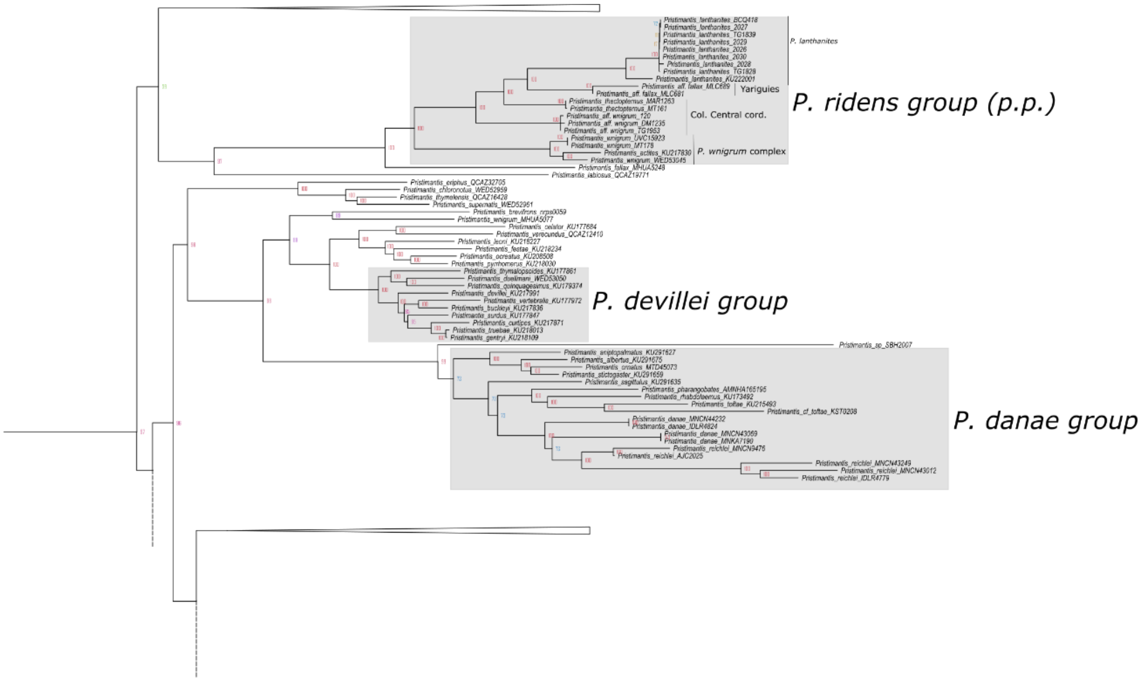

Figure 29. Clades with species previously placed in the *P. conspicillatus* group, with enough good taxonomic sampling and phylogenetic resolution to test the Andean diversification hypothesis.

Chapter 4. Conclusions and Future Research

Pristimantis conspicillatus group was recovered as a monophyletic under both parsimony and ML analysis. We provide a new definition of the *P. conspicillatus* group based on external morphology and cranial osteology. We have not yet found any morphological synapomorphy for the *P. conspicillatus* group. However, it is important to note that short palatines is a synapomorphy for a clade containing three species of the Atlantic Forest (*P. paulodutrai*, *P. ramagii* and *P. vinhai*), and *P. gaigei*. Similarity, the supra anal warts linking the species *P. fallax* and *P. lanthanites* was corroborated as synapomorphy (Lynch & Suarez-Mayorga, 2000).

The *P. conspicillatus* species group is an important component of the amphibian fauna of the Amazonian region. Their distribution range from Cordillera de la Costa and the Guyanas to Brazil (Atlantic forest and Mato Grosso), and Amazon basin of Colombia, Ecuador, Brazil and Peru. From Costa Rica to Bolivia, inhabit the Andes in Colombia, Ecuador, Peru and Bolivia. With the new definition of *P. conspicillatus* group members of this group are now principally distributed in Amazon basin and Andes slopes of Peru and Bolivia and few species are distributed on northern Andes. The *P. conspicillatus* species group distributed in Colombia are: *P. conspicillatus*, *P malkini*, *P. peruvianus*, and *P. vilarsi* (in the lowland tropical forest of Amazonian region); *P. achatinus* and *P. gaigei* in biogeographic region, and "*P. aff achatinus*" as the only species that occur in the Andean region at middle elevation of Cordillera Central and Occidental.

The sister taxon of *P. conspicillatus* group is a clade composed of species that occur in the Cordillera Oriental de Colombia, some of these species (*P. carranguerorum*, *P. medemi* and *P. savagei*) were initially allocated in the *P.*

conspicillatus group. The external morphology of the sister taxon species does not match the characteristics shared by the *P. conspicillatus* species group.

Our results identify Amazonia + North Andes as the most probable ancestral range of the most recent common ancestor of *P. conspicillatus* group. The highland species of *P. conspicillatus* group are more related to lowland species, where the montane species are derived and the basal species are confined to the lowlands, supporting the hypothesis of montane regions largely as 'species pumps' and lowland regions as 'museums'. Within the *P. conspicillatus* group there is not a generalized patter of speciation, ecological speciation and vicariance models explain the diversification of the *P. conspicillatus* group.

Our results corroborated that directionally of diversification in the genus *Pristimantis* is from the north to south Andes, we also identify the North Andes as an ancestral area of Pristimantis. Our results support the hypothesis that in Pristimantis species it is harder to adapt to the páramo respect to the lower bands.

We modified some morphological characters that has been proposed as diagnostic in terrarana. This study identify new lineages inside and outside of the *P. conspicillatus* group and this work detect two morphological synapomorphies and some morphological characters that are useful to diagnose clades. The sampling within the *P. conspicillatus* group was incomplete because we have not studied the morphology of all terminals in the *P. conspicillatus* clade. Therefore, is necessary complete our morphological matrix with a view to proposing more morphological characters that can be useful in diagnose some clades. Furthermore, we are going to make detailed study of some morphological characters (for example, shape of the squamosal ventral ramus) in order to find morphological synapomorphies. In addition, upon completion the morphological matrix we have more phenolic evidence to name the new lineage detected with molecular data.

It is clear that Amazon region has played a significant role in the evolution of *P. conspicillatus* group. We need future biogeographic analysis; taking into account other proposed that subdivide the Amazonian region in order to test the effect of the rivers in the diversification of Amazonian

References

Ab'Sáber, A.N. 1977. Os domínios morfoclimáticos na América do Sul. Geomorfologia. **52** , 1–23.

AmphibiaWeb: Information on amphibian iology and conservation (web aplication),

2006.Berkeley, California: AmphibiaWeb. Available at: http://amphibiaweb.org/

Andersson, F.E., Swofford, D.L., 2004. Should we be worried about long-branch attraction in real data sets? Investigations using metazoan 18S rDNA. Mol. Phyl. Evol. 33, 440–451.

Antonelli, A., Nylander, J. A .A, Persson, C., & Sanmartín, I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(24), 9749–54. http://doi.org/10.1073/pnas.0811421106

Arroyo, S. B. 2007. *New frog (Brachycephalidae: Eleutherodactylus) from the Western Flank of the Cordillera Oriental of Colombia*. Zootaxa 1389: 61–68.

Antonelli, A., Nylander, J. A. A., Persson, C. & Sanmartın, I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proceedings of the Natural Academy of Sciences, 106, 9749–9754.

Aspden, J. a., & Litherland, M. 1992. The geology and Mesozoic collisional history of the Cordillera Real, Ecuador. *Tectonophysics*, *205*(1-3), 187–204. http://doi.org/10.1016/0040-1951(92)90426-7

Ayala, R. C., G. Bayona, A. Cardona, C. Ojeda, O. C. Montenegro, C. Montes, V. Valencia, and C. Jaramillo. 2012. The paleogene synorogenic succession in the northwestern Maracaibo block: Tracking intraplate uplifts and changes in sediment delivery systems. Journal of South American Earth Sciences 39:93–111.

Barbour, T., and G. K. Noble. 1920. *Some amphibians from northwestern Peru, with a revision of the genera Phyllobates and Telmatobius*. Bulletin of the Museum of Comparative Zoology. Cambridge, Massachusetts 63: 395–427.

Barrio-Amorós, C.L. & Molina, C.R. 2006. A new Eleutherodactylus (Anura: rachycephalidae) from the Venezuelan Guayana, and redescription of Eleutherodactylus vilarsi (Melin). Zootaxa, 1302, 1–20.

Barry, D. & Hartigan, J. 1987 Statistical analysis of hominid molecular evolution. Statistical Science, 2, 191–210.

Batalha-Filho, H., Cabanne, G.S., Miyaki, C.Y., 2012. Phylogeography of an Atlantic Forest passerine reveals demographic stability through the last glacial maximum. Mol. Phylogenet. Evol. 65, 892–902.

Bayona, G., A. Cardona, C. Jaramillo, A. Mora, C. Montes, V. Caballero, H. Mahecha, F. Lamus, O. Montenegro, G. Jimenez, A. Mesa, and V. Valencia. 2013. Onset of fault reactivation in the Eastern Cordillera of Colombia and proximal Llanos Basin; response to Caribbean − South American convergence in early Palaeogene time. Geological Society, London, Special Publications.

Bernal MH, Lynch JD. 2013. Thermal tolerance in anuran embryos with different reproductive modes: relationship to altitude. The Scientific World Journal, 2013: 1-7.

Bergsten J. 2005. A review of long-branch attraction. Cladistics 21: 163–193.

Bleiweiss, R. 1998. Origin of hummingbirds. *Biological Journal of the Linnaean Society*, **63**: 77-97.

Boettger, O. 1892. Katalog der Batrachier-Sammlung im Museum der Senckenbergischen Naturforschenden Gesellshaft in Frankfurt am Main. Frankfurt a. M.: Gebrüder Knauer.

Bokermann, W. C. A. 1975 "1974". Três espécies novas de Eleutherodactylus do sudeste da Bahia, Brasil (Anura, Leptodactylidae). Revista Brasileira de Biologia 34: 11–18.

Bossuyt, F., Milinkovitch, M.C. 2001. Amphibians as indicators of early Tertiary Out-ofIndia dispersal of vertebrates. Science 292, 93–95.

Boulenger, G. A. 1888. On some reptiles and batrachians from Iguarasse, Pernambuco. Annals and Magazine of Natural History, Series 6, 2: 40–43.

Boulenger, G. A. 1898. An account of the reptiles and batrachians collected by Mr. W. F. H. Rosenberg in Western Ecuador. Proceedings of the Zoological Society of London 1898: 107–126.

Bush, M. B., & Oliveira, P. E. De. 2006.The rise and fall of the Refugial Hypothesis of Amazonian speciation: a paleoecological perspective. *Biota Neotropica.* http://doi.org/10.1590/S1676-06032006000100002

Caballero, V., M. Parra, and A. R. Mora Bohorquez. 2010. Levantamiento de la Cordillera Oriental de Colombia durante el Eoceno tardío-Oligoceno temprano: Proveniencia sedimentaria en el Sinclinal de Nuevo Mundo, Cuenca Valle Medio del Magdalena. Geologia Colombiana 32:45–77.

Camacho-Badani, T., M. H. Yánez-Muñoz, and S. R. Ron. 2012. Pristimantis citriogaster Duellman, 1992 (Amphibia: Craugastoridae): First record from Ecuador, altitudinal distribution extension with distribution map and phylogram. Check List. Journal of Species Lists and Distribution 8: 513–515.

Canedo, C. & Haddad, C.F.B. 2012. Phylogenetic relationships within anuran clade Terrarana, with emphasis on the placement of Brazilian Atlantic rainforest frogs genus Ischnocnema (Anura: Brachycephalidae). Molecular Phylogenetics and Evolution, 65, 610–620.

Carnaval, A.C., Hickerson, M.J., Haddad, C.F.B., Rodrigues, M.T., Moritz, C., 2009.Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science 323, 785–789.

Castroviejo-Fisher, S., Guayasamin, J.M Juan., Gonzalez-Voyer, A., Vila C. 2014. Neotropical diversification seen through glassfrogs. Journal of Biogeography, 41, 66–80.

Clarke, B. T. 1982. A new genus of ranine frog (Anura: Ranidae) from Somalia. Bulletin of the British Museum (Natural History). Zoology 43: 179–183.

Crawford, A.J. and Smith, E.N. 2005. Cenozoic biogeography and evolution in directdeveloping frogs of Central America (Leptodactylidae : Eleutherodactylus) as inferred from a phylogenetic analysis of nuclear and mitochondrial genes. Molecular Phylogenetics and Evolution, 35, pp

Catchpole, D. 2012. *Orographic gradients in climate and forest cover at the Cordillera Yanachaga , Peru*. University of Tasmania.

Cavalli-Sforza, L. L., & Edwards, A. W. F..1967. Phylogenetic analysis. Models and estimation procedures. *American Journal of Human Genetics*, *19*(3 Pt 1), 233–257. http://doi.org/10.2307/2406616

Cediel, F., & Shaw, R. P. 2003. Tectonic Assembly of the Northern Andean Block. *Eds., The Circum-Gulf of Mexico and the Caribbean: Hydrocarbon Habitats, Basin Formation, and Plate Tectonics*, *79*, 815–848.

Chapman, F. M. 1917. The distribution of bird-life in Colombia: a contribution to a biological survey of South America (Vol. 36). order of the trustees. *Bull. Am. Mus. Nat. Hist.*, *36*, 729.

Clough, M. & Summers, K. 2000. Phylogenetic s ystematics and biogeography of the poison frogs: evidence from mitochondrial DNA sequences. *Biological Journal of the Linnaean Society*, **70**: 515-540.

Coltorti, M., & Ollier, C. D. 2000. Geomorphic and tectonic evolution of the Ecuadorian Andes. *Geomorphology*, *32*(1-2), 1–19. http://doi.org/10.1016/S0169-555X(99)00036-7

Costa, L. P. 2003.The historical bridge between the Amazon and the Atlantic Forest of Brazil: A study of molecular phylogeography with small mammals. *Journal of Biogeography*, *30*(1), 71–86. http://doi.org/10.1046/j.1365-2699.2003.00792.x

Cuatrecasas, J. 1979. Comparación fitogeográfica de Páramos entre varias Cordilleras . In M. L. Salgado-Labouriau (Ed.), *El medio ambiente páramo. Actas del seminario de Mérida, Venezuela* (pp. 89–99). Caracas: Centro de Estudios Avanzados.

Dacks, J.B., Marinets, A., Doolittle, W.F., Cavalier-Smith, T.,Logsdon, J.M., 2002. Analyses of RNA polymerase II genes from free-living protists: phylogeny, long branch attraction, and the eukaryotic big bang. Mol. Biol. Evol. 19, 830–840.

Darst, C.R. & Cannatella, D.C. 2004. Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. Molecular Phylogenetics and Evolution, 31, 462–475.

De Oliveira, P. E., Barreto, A. M. F., & Suguio, K. .1999. Late Pleistocene/Holocene climatic and vegetational history of the Brazilian caatinga: The fossil dunes of the middle Sao Francisco River. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *152*(3-4), 319–337. http://doi.org/10.1016/S0031-0182(99)00061-9

De Queiroz, K., and S. Poe. 2001. Philosophy and phylogenetic inference: a comparison of likelihood and parsimony methods in the context of Karl R. Popper's writings on corroboration. Systematic Biology 50:305-321.

Descimon, H. 1986). Origin if lepidopterans faunas in the high tropical Andes. In F. Vuilleumier & M. Monasterio (Eds.), *High altitude tropical biogeography* (pp. 500–532). Oxford UP: New York: American Museum of Natural History.

Donoghue, M.J. & Moore, B.R. 2003.Toward an integrative historical biogeography. Integrative and Comparative Biology,43, 261–270.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Duellman, W.E. 1979. The Herpetofauna of the Andes; patterns of distribution, origin, differentiation, and present communities. Pp. 371- 460. In: Duellman, W.E. (Eds.) The South American Herpetofauna: Its origin, evolution, and dispersal. Monograph of the Museum of Natural History, University of Kansas. No 7. Pp. 1-485..536-555.

Duellman, W.E. 1963. Amphibians and reptiles of the rainforest of southern El Petén, Guatemala. University of Kansas Publications, Museum of Natural History, 15, pp.205249.

Duellman, W. E. 1992. A new species of the Eleutherodactylus conspicillatus group (Anura: Leptodactylidae) from northeastern Peru. Revista Española de Herpetología, 6, pp.23-29.

Duellman, W. E., and C. A. Toft. 1979. Anurans from the Serranía de Sira, Amazonian Perú: Taxonomy and biogeography. Herpetologica 35: 60–70.

Duellman, W.E. and Pramuk, J. 1999. Frogs of the genus Eleutherodactylus (Anura: Leptodactylidae) in the Andes of northern Peru. Scientific Papers Natural History Museum the University of Kansas, 13, pp.1-78.

Duellman, W. E., and S. B. Hedges. 2005. Eleutherodactyline frogs (Anura: Leptodactylidae) from the Cordillera Yanchaga in central Peru. Copeia 2005: 526–538.

Duellman, W. E., and S. B. Hedges. 2007. Three new species of Pristimantis (Lissamphibia, Anura) from montane forests of the Cordillera Yanachaga in central Peru. Phyllomedusa. Belo Horizonte 6: 119–135.

Duellman, W. E., and E. Lehr. 2007. Frogs of the genus Eleutherodactylus (Leptodactylidae) in the Cordillera Occidental in Peru with descriptions of three new species. Scientific Papers. Natural History Museum, University of Kansas 39: 1–13.

Duellman, W.E. & Lehr, E. 2009. Terrestrial–breeding frogs (Strabomantidae) in Peru. Nature und Tier Verlag, Münster, Germany, 384 pp.

Duméril, A.M.C.and Bibron, G., 1841. Erpétologie Générale ou Histoire Naturelle Compléte des Reptiles. Tome 8. Comprenant l'Histoire Générale des Batraciens, et la description des cinquante-deux generes et des cent soixante-trois espéces des deux premiers sous-ordres: Les péromèles qui n'ont pas de members, et les anoures qui sont privés de la queue. Librairie Encyclopédique de Roret, Paris, 792 p.

Dunn, E. R. 1931. New frogs from Panama and Costa Rica. Occasional Papers of the Boston Society of Natural History 5: 385–401.

Ebach, M.C., Humphries, C.J. & Williams, D.M. 2003. Phylogenetic biogeography deconstructed. Journal of Biogeography, 30, 1285–1296.

England, P., & Molnar, P.. 1990. Surface uplift, uplift of rocks, and exhumation of rocks. *Geology*, *18*(12), 1173–1177. http://doi.org/10.1130/0091-3(1990)018<1173:SUUORA>2.3.CO

Farris, J.S. 1983. The logical basis of phylogenetic analysis. In: Platnick, N.I. & Funk, V.A. (Eds.), Advances in Cladistics. Proceedings of the Second Meeting of the Willi Hennig Society, Volume 2. Columbia University Press, New York, pp. 7–36.

Felsenstein, J. 1973. Maximum likelihood and minimum steps methods for estimating evolutionary trees from data on discrete characters. Syst. Zool. 22: 240–249.

Felsenstein J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Systematic Zoology 27: 401–410.

Felsenstein, J. 1981. A likelihood approach to character weighting and what it tells us about parsimony and compatibility.

Felsenstein, J., 1985. Confidence limits on phylogenies – an approach using the bootstrap. Evolution 39, 783–791.

Felsenstein, J. 2004. Inferring phylogenies. Sinauer Associates, Inc., Sunderland. 664 p.

Flores, G., and L. O. Rodríguez. 1997. Two new species of the Eleutherodactylus conspicillatus group (Anura: Leptodactylidae) from Perú. Copeia 1997: 388–394.

Frost, Darrel R. 2015. Amphibian Species of the World: an Online Reference. Version 6.0 (Date of access). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA.

Fernandes, A. M., Gonzalez, J., Wink, M., & Aleixo, A. 2013. Multilocus phylogeography of the Wedge-billed Woodcreeper Glyphorynchus spirurus (Aves, Furnariidae) in lowland Amazonia: Widespread cryptic diversity and paraphyly reveal a complex diversification pattern. *Molecular Phylogenetics and Evolution*, *66*(1), 270–282. http://doi.org/10.1016/j.ympev.2012.09.033

Fouquet, A., Loebmann, D., Castroviejo-Fisher, S., Padial, J. M., Orrico, V. G. D., Lyra, M. L., Rodrigues, M. T. 2012. From Amazonia to the Atlantic forest: Molecular phylogeny of Phyzelaphryninae frogs reveals unexpected diversity and a striking biogeographic pattern emphasizing conservation challenges. *Molecular Phylogenetics and Evolution*, *65*(2), 547–561. http://doi.org/10.1016/j.ympev.2012.07.012

Fundación Pro - Sierra Nevada De Santa Marta. 2015. (Online) Disponible en: http://www.prosierra.org/ (Access 25/01/2015) Fundación Pro - Sierra Nevada De Santa Marta. 2015. Referencia online

Funk, W. C., Caldwell, J. P., Peden, C. E., Padial, J. M., De la Riva, I., & Cannatella, D. C. 2007. Tests of biogeographic hypotheses for diversification in the Amazonian forest frog, Physalaemus petersi. *Molecular Phylogenetics and Evolution*, *44*(2), 825–837. http://doi.org/10.1016/j.ympev.2007.01.012

Garcia-Moreno, J., Arctander, P. & Fjeldsa, J. 1998. Pre-Pleistocene differentiation among chat-tyrants. Condor, 100, 629–640.

Garcia-Moreno, J., Arctander, P. & Fjeldsa, J. 1999. Strong diversification at the treeline among metallura hummingbirds.Auk, 16, 702–711.

Garzione, C. N., Hoke, G. D., Libarkin, J. C., Withers, S., MacFadden, B., Eiler, J. Mulch, A. 2008. Rise of the Andes. *Science (New York, N.Y.), 320*(5881), 1304–7. http://doi.org/10.1126/science.1148615

Garzón-Orduña, I. J., Benetti-Longhini, J. E., & Brower, A. V. Z.. 2014.Timing the diversification of the Amazonian biota: butterfly divergences are consistent with

Pleistocene refugia. *Journal of Biogeography*, n/a–n/a. http://doi.org/10.1111/jbi.12330

Gascon, C., Lougheed, S. C., & Bogart, J. P.. 1996.Genetic and morphological variation in Vanzolinius discodactylus: A test of the river hypothesis of speciation. *Biotropica*, *28*(3), 376–387. http://doi.org/10.2307/2389201

Gengler–Nowak, K. 2002.. Reconstruction of the Biogeographical History of Malesherbiaceae. *The Botanical Review*, *68*(1), 171–188. http://doi.org/10.1663/0006-8101(2002)068[0171:ROTBHO]2.0.CO;2

Gentry, A. H. 1982. Neotropical floristic diversity : phytogeographical connections between central and south america , pleistocene climatic fluctuations , or an accident of the andean orogeny. *Annals of Missouri Botanical Garden*, *69*(3), 557–593.

Gibbs A.K. & Barron, C.N. 1993. *The geology of the Guiana Shield*. Oxford University Press, New York.

Graham, A. 2009. The Andes: a Geological Overview From a Biological Perspective. *Annals of the Missouri Botanical Garden*, *96*(3), 371–385. http://doi.org/10.3417/2007146

Gregory-Wodzicki, K. M. 2000. Uplift history of the Central and Northern Andes: A review. *Bulletin of the Geological Society of America*, *112*(7), 1091–1105. http://doi.org/10.1130/0016-7606(2000)112<1091:UHOTCA>2.0.CO;2

Goin, C. J., and D. M. Cochran. 1963. Two new genera of leptodactylid frogs from Colombia. Proceedings of the California Academy of Sciences, 4th Series, 31, pp.499505.

Goldman, N. 1990. Maximum likelihood inference of phylogenetic trees, with special reference to a poisson

process model of DNA substitution and to parsimony analysis. Systematic Zoology 39:345-361

Goloboff P.A. 1999. Analyzing large data sets in reasonable times: Solutions for composite optima. Cladistics 15:415–428.

Goloboff P.A., Farris J.S., Nixon K.C. 2008. TNT, a free program for phylogenetic analysis. Cladistics 24:774–786.

Grant, T., D. R. Frost, J. P. Caldwell, R. Gagliardo, C. F. B. Haddad, P. J. R. Kok, D. B. Means, B. P. Noonan, W. E. Schargel, and W. C. Wheeler. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). Bulletin of the American Museum of Natural History 299:1–262

Guarnizo CE, Werneck FP, Giugliano LG, Santos MG, Fenker J, Sousa L,D'Angiolella AB, dos Santos AR, Strüssmann C, Rodrigues MT. 2015. Cryptic lineages and diversification of an endemic anole lizard (squamata,dactyloidae) of the cerrado hotspot. Mol Phylogenet Evol. 2016;94(Part A):279–89.

Guayasamin, J. M. 2004. The Eleutherodactylus orcesi species group (Anura:Leptodactylidae): comparative osteology and comments on its monophyly. Herpetological Monographs, 18, pp.142-174.

Günther, A. C. L. G. 1858. Neue Batrachier in der Sammlung des britischen Museums. Archiv für Naturgeschichte. Berlin 24: 319–328.

Haffer, J. 1969. Speciation in Amazonian Forest Birds. *Science*, *165*(3889), 131–137.

Henning, W.1965. Phylogenetic systematics. *Annual Review of Entomology*, *10*(1), 97–116.

Hooghiemstra, H., & Van Der Hammen, T. 1998. Neogene and Quaternary development of the neotropical forest: the fores refugia hypothesis, and a literature overview. *Earth-Science Reviews*, *44*, 147–183.

Hoorn, C., Guerrero, J., Sarmiento, G. A., & Lorente, M. A. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology*. http://doi.org/10.1130/0091-7613(1995)023<0237:ATAACF>2.3.CO;2

Hoorn, C., Wesselingh, F.P., Ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartin, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Sarkinen, T. & Antonelli, A. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330: 927–931.

Hughes, C., & Eastwood, R. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(27), 10334–9. http://doi.org/10.1073/pnas.0601928103

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis. Nucl. Ac. Symp. Ser. 41, 95–98.

Hanken, J., M.W. Klymkowsky, C.H. Summers, D.W. Seufert, and N. Ingebrigtsen. 1992. Cranial ontogeny in the direct-developing frog, Eleutherodactylus coqui (Anura: Leptodactylidae), analyzed using whole-mount immunohistochemistry. J. Morphol., 211, pp.95-118.

Hedges, S.B.1989. Evolution and biogeography of West Indian frogs of the genus Eleutherodactylus: slow-evolving loci and the major groups. In: Woods, C.A., ed. Biogeography of the West Indies: Past, Present, and Future. Sandhill Crane Press, Gainesville, Florida, pp. 305-370.

Hedges, S.B., Duellman, W.E. & Heinicke, M.P. 2008. New World direct-developing frogs (Anura, Terrarana), molecular phylogeny, classification, biogeography, and conservation. Zootaxa, 1737, 1–182

Heinicke M.P, Duellman W.E, Hedges S.B. 2007. Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. Proceedings of the National Academy of Sciences of the USA, 104, pp.10092–10097.

Henle, K. 1992. Zur Amphibienfauna Perus nebst Beschreibung eines neuen Eleutherodactylus (Leptodactylidae). Bonner Zoologische Beiträge 43: 79–129.

Heyer, W. R., and A. M. Muñoz. 1999. Validation of Eleutherodactylus crepitans Bokermann, 1965, notes on the types and type locality of Telatrema heterodactylum Miranda-Ribeiro, 1937, and a description of a new species of Eleutherodactylus from Mato Grosso, Brazil (Amphibia: Anura: Leptodactylidae). Proceedings of the Biological Society of Washington 112: 1–18.

Hillis, D.M., Bull, J.J., 1993. An empirical-test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182–192.

Hillis, D.M.and de Sá, R.O.1988. Phylogeny and taxonomy of the Rana palmipes group (Salientia: Ranidae). Herpetological Monographs, 2, pp.1-26.

Hooghiemstra, H., van der Hammen, T. & Cleef, A. 2000. Evolution of forests in the northern Andes and Amazonian lowlands during the Tertiary and Quaternary. In M. Guariguata (Ed) Ecology of Neotropical Rainforests. The Netherlands: Leerstoelgroep Resource Ecology, Wageningen University.

Hoogmoed, M. S., J. D. Lynch, and J. Lescure. 1977. A new species of Eleutherodactylus from Guiana (Leptodactylidae, Anura). Zoologische Mededelingen. Leiden 51: 33–41.

Huelsenbeck, J. P., B. Ronquist, R. Nielsen, and J. P. Bollback. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294:2310-2314.

Jaillard, E., Hérail, G., Monfret, T., Díaz-Martínez, E., Baby, P., Lavenu, A. & Dumont, J.F. 2000. Tectonic evolution of the Andes of Ecuador, Peru, Bolivia and northernmost Chile. *Tectonic evolution of South America* (ed. by U.G. Cordani, E.J. Milani, A. Thomaz-Filho and D.A. Campos), pp. 481–559. Brazilian Academy of Science, Rio de Janeiro.

Jaramillo, C., Rueda, M. J., & Mora, G.. 2006. Cenozoic plant diversity in the neotropics. *Science (New York, N.Y.)*, *311*(5769), 1893–6. http://doi.org/10.1126/science.1121380

Jiménez de la Espada, M. (1871 "1870") Faunae neotropicalis species quaedam nondum cognitae. Jornal des Sciencias Mathematicas Physicas e Naturales, Academia Real das Sciencias de Lisboa, 3, 57–65.

Kaiser, H., C. M. Dwyer, W. Feichtinger, and M. Schmid. 1995. A new species of Eleutherodactylus (Anura: Leptodactylidae) from Tobago, West Indies and its morphometric and cytogenetic characterization. Herpetological Natural History 3: 151–163.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. 2005. MAFFT, a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30, 3059–3066

Kimura, M. 1977. Preponderance of synonymous changes as evidence for the neutral theory of molecular evolution. *Nature*, *267*(5608), 275–276.

Kohn, B. P., Shagam, R., & Subieta, T. 1984. Results and preliminary implications of sixteen fission-track ages from rocks of the western Caribbean mountains, Venezuela. *Geological Society of America Memoirs*, *162*, 415–422.

Kroonenberg, S., Bakker, J., & Van der Wiel, M.. 1990. Late Cenozoic uplift and paleogeography of the Colombian Andes: constraints on the development of high-andean biota. *Geologie*, (69), 279–290.

Kaiser, H., C. M. Dwyer, W. Feichtinger, and M. Schmid. 1995. A new species of Eleutherodactylus (Anura: Leptodactylidae) from Tobago, West Indies and its morphometric and cytogenetic characterization. Herpetological Natural History 3: 151–163.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. 2005. MAFFT, a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30, 3059–3066

Kieswetter, C. M., and C. J. Schneider. 2013. Phylogeography in the northern Andes: Complex history and cryptic diversity in a cloud forest frog, Pristimantis w-nigrum (Craugastoridae). Molecular Phylogenetics and Evolution 69: 417–429.

Kluge, A. G. and Wolf, A. J. 1993. Cladistics: What‟s in a word?, Cladistics, 9, pp.183– 199.

Kluge, A.G. & Grant, T. 2006. From conviction to anti–superfluity, old and new justifications for parsimony in phylogenetic inference. Cladistics, 22, 276–288.

Köhler, J., and K.-H. Jungfer. 1995. Eine neue Art und ein Erstnachweis von Fröschen der Gattung Eleutherodactylus aus Bolivien. Salamandra 31: 149–156.

Kück P, Mayer C, Wagele J-W, Misof B. 2012. Long branch effects distort maximum likelihood phylogeniesin simulations despite selection of the correct model. PLoS One. 7:1–7.

Lartillot N, Philippe H. 2004. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. Mol Biol Evol. 21:1095–109.

Lartillot N, Brinkmann H, Philippe H. 2007. Suppression of long-branch attraction artefacts in the animalphylogeny using a site-heterogeneous model. BMC Evol Biol. 7 Suppl 1:1–14.

Latrubesse, E.M., Cozzuol, M., Da Silva-Caminha, S.A.F., Rigsby,C.A., Absy, M.L. & Jaramillo, C. 2010. The Late Miocene paleogeography of the Amazon Basin and the evolution of the Amazon River system. *Earth Sci. Rev.* 99: 99–124.

Le Saout S, Hoffmann M, Shi Y, Hughes A, Bernard C, Brooks TM, Bertzky B, Butchart SHM, Stuart SN, Badman T, Rodrigues ASL. 2013. Protected areas and effective biodiversity conservation. Science, 342, 803–805.

Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data.Systematic Biology 50:913-925.

Lougheed, S. C., Gascon, C., Jones, D. a, Bogart, J. P., & Boag, P. T. 1999. Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog (Epipedobates femoralis). *Proceedings. Biological Sciences / The Royal Society*, *266*(1431), 1829–1835. http://doi.org/10.1098/rspb.1999.0853

Lutz, H. L., Weckstein, J. D., Patané, J. S. L., Bates, J. M., & Aleixo, A. 2013. Biogeography and spatio-temporal diversification of Selenidera and Andigena Toucans (Aves: Ramphastidae). *Molecular Phylogenetics and Evolution*, *69*(3), 873–883. http://doi.org/10.1016/j.ympev.2013.06.017

Lynch, J. D. 1986. Origins of the high Andean herpetological fauna. In *High altitude tropical biogeography* (pp. 478–499).

La Marca, E. 2004. Descripción de dos nuevos anfibios del piedmemonte andino de Venezuela. Herpetotropicos. Mérida, Venezuela 1: 1–9.

Lehr, E., and W. E. Duellman. 2007. Two new species of Eleutherodactylus (Anura: Leptodactylidae) from the Cordillera Occidental in Peru. Copeia 2007: 140–149.

Luebert, F., Hilger, H. H. & Welgend, W. 2011. Diversification in the Andes: age and origins of South American Heliotropium lineages (heliotropiaceae, boraginales). Molecular Phylogenetics and Evolution, 61, 90–102.

Lynch, J.D. 1964. A review of the Frogs of the Mexicanus and Rhadophis Groups (Genus Eleutherodactylus) and the Evolution of the Genus in Northern Central America. Msc. University of Illinois.

Lynch, J. D. 1965. Sacral fusions in some neotropical Rana. Trans. Illinois Acad. Sci., 58, pp.195-199.

Lynch, J. D. 1968. Systematic status of some Andean leptodactylid frogs with a description of a new species of Eleutherodactylus. Herpetologica, 24, pp.289-300.

Lynch, J. D. 1969. Taxonomic notes on Ecuadorian frogs (Leptodactylidae: Eleutherodactylus). Herpetologica, 25, pp.262-274.

Lynch, J.D. 1971. Evolutionary relationships, osteology, and zoogeography of leptodactyloid frogs. University of Kansas Museum of Natural History, Miscellaneous Pulications, 53, pp.1-238.

Lynch, J. D. 1975. The identity of the frog Eleutherodactylus conspicillatus (Günther), with descriptions of two related species from northwestern South America (Amphibia, Leptodactylidae). Contributions in Science. Natural History Museum of Los Angeles County, 272, pp.1-19.

Lynch, J. D. 1976. Three new leptodactylid frogs (genus Eleutherodactylus) from the Andean slopes of Colombia and Ecuador. Herpetologica, 32, pp.310-317.

Lynch, J. D. 1977. A new frog (Leptodactylidae: Eleutherodactylus) from the Pacific lowlands of Ecuador. Copeia 1977: 282–284.

Lynch, J. D. 1977. A new species of Eleutherodactylus from the Cordillera Occidental of Colombia (Amphibia: Anura: Leptodactylidae). Occasional Papers of the Museum of Zoology, University of Michigan 678: 1–6.

Lynch, J. D. 1979. A new frog species of the Eleutherodactylus fitzingeri group from the Pacific Andean versant in Ecuador. Herpetologica 35: 228–233.

Lynch, J. D. 1980. A taxonomic and distributional synopsis of the Amazonian frogs of the genus Eleutherodactylus. American Museum Novitates 2696: 1–24.

Lynch, J.D. 1981. The systematic status of Amblyphrynus ingeri (Amphibia: Leptodactyidae) with the description of an allied species in western Colombia. Caldasia, 13, pp.313–332.

Lynch, J. D. 1981 (b) Leptodactylid frogs of the genus Eleutherodactylus in the Andes of northern Ecuador and adjacent Colombia. Miscellaneous Publication. Museum of Natural History, University of Kansas, 72, pp.1-46.

Lynch, J. D. 1984. A new species of Eleutherodactylus (Amphibia: Anura: Leptodactylidae) from southern Andean Colombia. Herpetologica, 40, pp.234-237.

Lynch, J. D. 1986. A new species of broad-headed Eleutherodactylus from the Cordillera Occidental of Colombia (Amphibia, Leptodactylidae). Caldasia, 15, pp.503-509.

Lynch, J.D.1986. The definition of the Middle American clade of Eleutherodactylus based on jaw musculature (Amphibia: Leptodactyliodae). Herpetologica, 42, pp.248-258.

Lynch, J.D. 1989. The gauge of speciation: on the frequency of modes of speciation. In: D. Otte and J. A. Endler, eds, Speciation and its consequences. Sinauer Associates, Sunderland, MA. pp. 527–553.

Lynch, J. D. 1989. Intrageneric relationships of mainland Eleutherodactylus (Leptodactylidae). I. A review of the frogs assigned to the Eleutherodactylus discoidalis species group. Contributions in Biology and Geology. Milwaukee Public Museum, 79, pp.1-25.

Lynch, J. D. 1994. Two new species of the Eleutherodactylus conspicillatus group (Amphibia: Leptodactylidae) from the Cordillera Oriental of Colombia. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 19: 187–193.

Lynch, J. D. 1995. Three new species of Eleutherodactylus (Amphibia: Leptodactylidae) from the paramos of the Cordillera Occidental of Colombia. Journal of Herpetology, 29, pp.513-521.

Lynch, J. D. 1996. New frog (Eleutherodactylus: Leptodactylidae) from the Andes of eastern Colombia, part of a remarkable pattern of distribution. Copeia, 1996, pp.103-108.

Lynch, J.D. 1997. Ranas pequeñas, la geometría de la evolución , y la especiacion en los Andes colombianos. Rev. Acad. Colomb.Cienc, 23, pp.143-159.

Lynch, J.D. 1999. Intrageneric relationships of mainland Eleutherodactylus (Leptodactylidae). II. A review of Eleutehrodactylus sulcatus group. Rev. Acad. Colomb.Cienc, 21, pp.353-372.

Lynch, J.D. 2001. Four ostelogical synapomorphies within Eleutherodactylus (Amphia: Leptodactylidae) and their bearing on subgeneric classifications. Rev. Acad. Colomb.Cienc. 25 (94), pp.127-136.

Lynch, J. D. 2003. A new species of frog from northeastern Colombia (Genus Eleutherodactylus: Leptodactylidae). Rev. Acad. Colomb.Cienc, 27, pp.287-289.

Lynch, J. D. 2003. New species of frogs (Eleutherodactylus: Leptodactylidae) from the Cordillera Oriental of Norte de Santander and Santander, Colombia. Rev. Acad. Colomb.Cienc. 27: 449–460.

Lynch, J.D. and Hoogmoed, 1977: Two new species of Eleutherodactylus (Amphibia: Leptodactylidae) from northeastern South America. Proceedings of the Biological Society of Washington, 90, pp.424-439.

Lynch, J. D., and W. E. Duellman. 1980. The Eleutherodactylus of the Amazonian slopes of the Ecuadorian Andes (Anura: Leptodactylidae). Miscellaneous Publication. Museum of Natural History, University of Kansas 69: 1–86.

Lynch, J.D. and Myers, C.W. 1983. Frogs of the fitzingeri group of Eleutherodactylus in eastern Panama and Chocoan South America (Leptodactylidae). Bulletin of the American Museum of Natural History, 175, pp.481–572.

Lynch, J. D., and Ruiz-Carranza, P. M. 1985. A synopsis of the frogs of the genus *Eleutherodactylus* from the Sierra Nevada de Santa Marta. Occasional Papers of the Museum of Zoology, University of Michigan, 711, pp.1-59.

Lynch, J. D. and Burrowes, P. A. 1990. The frogs of the genus *Eleutherodactylus* (family leptodactylidae) at the La Planada reserve in southwestern Colombia with descriptions of eight new species. Occasional papers of the Museum of Natural History, University of Kansas, 136, pp.1-31.

Lynch, J.D. and Duellman, W. E. 1997. Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in western Ecuador: Systematycs, Ecology, and Biogeography. The University of Kansas Natural History Museum, Special Publications, 23, pp.1-236.

Lynch, J. D., and J. V. Rueda-Almonacid. 1999. New species of frogs from low and moderate elevations from the Caldas transect of the eastern flank of the Cordillera Central. Rev. Acad. Colomb.Cienc. 23: 307–314.

Lynch, J. D., Ruiz-Carranza, P. M. and Ardila Robayo, M. C. 1996. Three new species of *Eleutherodactylus* (Amphibia: Leptodactylidae) from high elevations of the Cordillera Central of Colombia. Caldasia, 18(3), pp.329–342.

Lynch, J. D., and Á. M. Suárez-Mayorga. 2000. A new frog (*Eleutherodactylus*: Leptodactylidae) from the southern part of the Cordillera Oriental of Colombia.

Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 24: 289–293.

Mégard, F. (1987) Cordilleran and marginal Andes: a review of Andean geology north of the Arica elbow (18°S). *Circum-Pacific orogenic belts and evolution of the Pacific Ocean Basin* (ed. by J.W.H. Monger and J. Francheteau), pp. 71–95. American Geophysical Union: Geodynamic Series 18, Washington, DC.

Mendoza, Á. M., Ospina, O. E., Cárdenas-Henao, H., & García-R, J. C. (2015). A likelihood inference of historical biogeography in the world's most diverse terrestrial vertebrate genus: Diversification of direct-developing frogs (Craugastoridae: *Pristimantis*) across the Neotropics. *Molecular Phylogenetics and Evolution*, *85*, 50–58. http://doi.org/10.1016/j.ympev.2015.02.001

Moler, R.C. Drewes, R.A. Nussbaum, J.D. Lynch, D. Green, and W.C. Wheeler. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History, 297, pp.1370.

Mora, A., Parra, M., Strecker, M. R., Sobel, E. R., Hooghiemstra, H., Torres, V., & Jaramillo, J. V. (2008). Climatic forcing of asymmetric orogenic evolution in the Eastern Cordillera of Colombia. *Bulletin of the Geological Society of America*, *120*(7-8), 930–949. http://doi.org/10.1130/B26186.1

Maciel, N. M., W. Vaz-Silva, R. M. de Oliveira, and J. M. Padial. 2012. A new species of Pristimantis (Anura: Strabomantidae) from the Brazilian Cerrado. Zootaxa 3265: 43–56.

Mau, B. 1996. Bayesian phylogenetic inference via Markov Monte Carlo Methods. Ph.D. dissertation., University of Wisconsin.

Melin, D. E. 1941. Contributions to the knowledge of the Amphibia of South America. Göteborgs Kungl. Vetenskaps-och Vitterhets-samhälles. Handlingar. Serien B, Matematiska och Naturvetenskapliga Skrifter 1: 1–71.

Mello CL, Metelo CMS, Suguio K, Kohler HC. 1999. Quaternary sedimentation, neotectonics and the evolution of the Doce River middle valley lake system (southeastern Brazil). Revista do Instituto Geol_ogico, 20, 29–36.

Mueses-Cisneros, J. J. 2006. A new species of *Eleutherodactylus* (Amphibia: Anura: Brachycephalidae) from the western flank of the Cordillera Oriental of Colombia. Zootaxa 1271: 29–35.

Mulch, A., Uba, C.E., Strecker, M.R., Schoenberg, R., Chamberlain, C.P., 2010. Late Miocene climate variability and surface elevation in the central Andes. Earth Planet. Sci. Lett. 290, 173–182.

Murphy, J. C. 1997. Amphibians and Reptiles of Trinidad and Tobago. Malabar, Florida: Krieger Publishing Company.

Myers,C.W. and Duellman, W.E. 1982. A new species of Hyla from Cerro Colorado, other tree frog records and geographical notes from western Panama. Am. Mus. Novitates, 2752, pp.1-32.

Nei M & Kumar S. **2000**. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

Noble, G.K. 1926. The pectoral girdle of the Brachycephalid frogs. American Museum Novitates, 230, pp.1–14.

Ochoa, D., Hoorn, C., Jaramillo, C., Bayona, G., Parra, M., & Parra, F. De. 2012. Journal of South American Earth Sciences The final phase of tropical lowland conditions in the axial zone of the Eastern Cordillera of Colombia : Evidence from three palynological records. *Journal of South American Earth Sciences*, *39*, 157–169. http://doi.org/10.1016/j.jsames.2012.04.010

Oliveira-Filho, A.T., Fontes, M.A.L., 2000. Patterns of floristic differentiation among Atlantic forests in southeastern Brazil and the influence of climate. Biotropica 32, 793–810

Padial, J. M., Chaparro, J. C., Castroviejo-Fisher, S., Guayasamin, J. M., Lehr, E., Delgado, A. J., Vaira, M., Teixera Jr., M., Aguayo, R., De la Riva, I. 2012. A revision of species diversity in the Neotropical genus Oreobates (Anura: Strabomantidae), with the description of three new species from the Amazonian slopes of the Andes. American Museum Novitates 3752:1-55.

Padial, J. M., & De La Riva, I.. 2009. Integrative taxonomy reveals cryptic Amazonian species of Pristimantis (Anura: Strabomantidae). *Zoological Journal of the Linnean Society*, *155*(1), 97–122. http://doi.org/10.1111/j.1096-3642.2008.00424.x

Parenti, L..2006. Common cause and historical biogeography.Biogeography in a changing world (ed. by M.C.Ebach and R.S. Tangney), pp. 61–71. CRC Press, Boca Raton, FL.

Parks S, Goldman N. 2014. Maximum likelihood inference of small trees in the presence of long branches.Syst Biol. 63(5):798-811

Parra, M., Mora, A., Jaramillo, C., Strecker, M. R., Sobel, E. R., Quiroz, L., Torres, V. 2009 Orogenic wedge advance in the northern Andes: Evidence from the Oligocene-Miocene sedimentary record of the Medina Basin, Eastern Cordillera, Colombia. *Bulletin of the Geological Society of America*, *121*(5-6), 780–800. http://doi.org/10.1130/B26257.1

Patel, S., Weckstein, J. D., Patané, J. S. L., Bates, J. M., & Aleixo, A. 2011. Temporal and spatial diversification of Pteroglossus araçaris (AVES: Ramphastidae) in the neotropics: Constant rate of diversification does not support an increase in radiation during the Pleistocene. *Molecular Phylogenetics and Evolution*, *58*(1), 105–115. http://doi.org/10.1016/j.ympev.2010.10.016

Pennington, R., & Dick, C. 2010. Diversification of the Amazoninan flora and its relation to key geological an enviromental events: a molecular perspective. In C. Hoorn & F. P. Wesselingh (Eds.), *Amazonia, Landscape and Species Evolution Species Evolution* (pp. 373–385). Wiley-Blackwell.

Pinto-Sánchez, N. R., Ibáñez, R., Madriñán, S., Sanjur, O. I., Bermingham, E., & Crawford, A. J. 2012. The Great American Biotic Interchange in frogs: Multiple and early colonization of Central America by the South American genus Pristimantis (Anura: Craugastoridae). *Molecular Phylogenetics and Evolution*, *62*(3), 954–972. http://doi.org/10.1016/j.ympev.2011.11.022

Pinto-da-Rocha, R., da Silva, M.B., Bragagnolo, C., 2005. Faunistic similarity and historic biogeography of the harvestmen of southern and southeastern Atlantic rain forest of Brazil. J. Arachnol. 33, 290–299.

Palumbi, S., Martin, A., Romano, S., McMillan, W., Stice, L., Grabowski, G., 1991. The Simple Fool‟s Guide to PCR, Version 2.0, Privately Published, Univ. Hawaii.

Padial, J. M., S. Castroviejo-Fisher, J. Köhler, E. Domic, and De la Riva, I. 2007. Systematics of the Eleutherodactylus fraudator species group (Anura: Brachycephalidae). Herpetological Monographs, 2, pp.213-240.

Padial, J.M. and De la Riva, I. 2009. Integrative taxonomy reveals cryptic Amazonian species of Pristimantis (Anura). Zoological Journal of the Linnaean Society, 155, pp.97122.

Padial, J. M., Castroviejo-Fisher, S., Köhler, Jörn, Vilà, C., Chaparro, J. C. and De la Riva, I. 2009. Deciphering the products of evolution at the species level: the need for an integrative taxonomy. Zoologica Scripta, 38: 431–447.

Padial, J. M., T. Grant, and D. R. Frost. 2014. Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria . Zootaxa 3825: 1–132

Posada, D. & Crandall, K.A. 1998. Modeltest, testing the model of DNA substitution. Bioinformatics, 14, 817–818.

Pyburn, W. F., and J. D. Lynch. 1981. Two little-known species of Eleutherodactylus (Amphibia: Leptodactylidae) from the Sierra de la Macarena, Colombia. Proceedings of the Biological Society of Washington 94: 404–412.

Pyron, R.A., Wiens, J.J., 2011. A large-scale phylogeny of Amphibia including over 2800 species and a revised classification of extant frogs, salamanders, and caecilians. Mol. Phylogenet. Evol. 61, pp.543–583.

Rannala B., Yang Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of molecular evolution 43:304-311.

Ree, R.H.; Sanmartín, I. (2009) Guest Editorial: Prospects and challenges for parametric models in historical biogeographical inference. *Journal of Biogeography* 36: 1211-1220.

Reynolds, R. P. and Icochea J. 1997. Amphibians and reptiles of the upper Rio Comainas, Cordillera del Condor. Pages 82-86, 202-206 in Thomas S. Schulenberg, Kim Awbrey, and Glenda Fabregas, editors. The Cordillera del Condor region of Ecuador and Peru: A Biological Assessment. RAP working papers No. 7. Conservation International, Washington, DC. 231 pp.

Ribas, C. C., Aleixo, A., Nogueira, A. C. R., Miyaki, C. Y., & Cracraft, J. 2012. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings. Biological Sciences / The Royal Society*, *279*(1729), 681–9. http://doi.org/10.1098/rspb.2011.1120

Richardson, J. E., Pennington, R. T., Pennington, T. D., & Hollingsworth, P. M. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science (New York, N.Y.)*, *293*(5538), 2242–5. http://doi.org/10.1126/science.1061421

Ring, U., Glodny, J., Will, T., & Thomson, S. 2010. The Hellenic Subduction System: High-Pressure Metamorphism, Exhumation, Normal Faulting, and Large-Scale Extension. *Annual Review of Earth and Planetary Sciences*, *38*(1), 45–76. http://doi.org/10.1146/annurev.earth.050708.170910

Rieppel, O.C. and Kearney, M. 2002. Similarity. Biol. J. Linn. Soc., 75, pp. 59-82.

Rivero, J. A. 1961. Salientia of Venezuela. Bulletin of the Museum of Comparative Zoology. Cambridge, Massachusetts 126: 1–207.

Rivero, J. A., and M. A. Serna. 1988 "1987". Tres nuevas especies de Eleutherodactylus (Amphibia, Leptodactylidae) de Antioquia, Colombia. Caribbean Journal of Science 23: 386–399.

Ronquist, F. 1997. Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography.Systematic Biology, 46, 195–203.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes version 3.1: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Rambaut, A., Drummond, A., 2004. Tracer 1.3. University of Edinburgh, Edinburgh,UK <http://tree.bio.ed.ac.uk/software/tracer>.

Ruthven, A. G. 1922. The amphibians and reptiles of the Sierra Nevada de Santa Marta, Colombia. Miscellaneous Publications. Museum of Zoology, University of Michigan 8: 1– 69.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Särkinen, T., Pennington, R. T., Lavin, M., Simon, M. F., & Hughes, C. E. 2012. Evolutionary islands in the Andes: Persistence and isolation explain high endemism in Andean dry tropical forests. *Journal of Biogeography*, *39*(5), 884–900. http://doi.org/10.1111/j.1365-2699.2011.02644.x

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular Cloning: a Laboratory Manual. Cold

Sankoff, D.. 1975. Minimal Mutation Trees of Sequences. *SIAM Journal on Applied Mathematics.*

Sanmartín, I. 2014. Biogeography. In: Vargas; Zardoya (eds.).The Tree of Life. Sinauer Associates, Inc., Publishers, Sunderland, US. pp. 156-166.

Sanmartin, I.; van der Mark, P.; Ronquist, F. 2008. Inferring dispersal: a Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. *Journal of Biogeography 35*:428-449.

Sanmartín, I.; Enghoff, H; Ronquist, F. 2001. Patterns of animal dispersal, vicariance, and diversification in the Holarctic. *Biological Journal of the Linnean Society 73*: 345-390

Santos, J. C., Coloma, L. a, Summers, K., Caldwell, J. P., Ree, R., & Cannatella, D. C. 2009. Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. *PLoS Biology*, *7*(3), e56. ttp://doi.org/10.1371/journal.pbio.1000056

Savage, J. M. 1975. Systematics and distribution of the Mexican and Central American stream frogs related to Eleutherodactylus rugulosus. Copeia, 1975(2), pp.254-306.

Savage, J.M. 1987. Systematics and distributions of the Mexican and Central American rainfrogs of the Eleutherodactylus gollmeri group (Amphbia: Leptodactylidae). Fieldiana Zoology (new ser), 33, pp.1-57.

Savage, J,M. and Heyer, W.R. 1969. The tree-frogs (Family Hylidae) of Costa Rica: diagnósis and distribution. Rev. Biol Trop. 16(1), pp.1-127.

Sedano, R. E., & Burns, K. J. 2010. Are the Northern Andes a species pump for Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves: Thraupini). *Journal of Biogeography*, *37*(2), 325–343. http://doi.org/10.1111/j.1365-2699.2009.02200.x

Siddall M. E, Kluge A. G. 1997. Probabilism and phylogenetic inference. Cladistics 13:313-336.

Simon, M., Grether, R., de Queiroz L.P., Skema C., Pennington, R.T. & C.E. Hughes. 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in-situ evolution of adaptations to fire. Proceedings of the National Academy of Sciences, USA 106: 20359-20364.

Schulte, J. A., Macey, J. R., Espinoza, R. E. & Larson, A. 2000. Phylogenetic relationships in the iguanid lizard genus Liolaemus: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. Biological Journal of the Linnean Society, 69, 75–102. Spring Harbor Laboratory Press, New York, USA.

Schlüter, A., and D. Rödder. 2007 "2006". Three new frogs of the genus Eleutherodactylus (Amphibia, Leptodactylidae) from Guaiquinima Table Mountain, Bolivar, Venezuela. Herpetotropicos. Mérida, Venezuela 3: 88–99.

Siqueira, S., Jr., O. Aguiar, Jr., C. Strüssmann, M. L. Del Grande, and S. M. Recco-Pimentel. 2008. Chromosomal analysis of three Brazilian "eleutherodactyline" frogs (Anura: Terrarana), with suggestion of a new species. Zootaxa 1860: 51–59.

Sober, E. 1981. The principle of parsimony. Br. J. Phil.Sci. 32: 145–156.

Steindachner, F. 1864. Batrachologische Mittheilungen. Verhandlungen des ZoologischBotanischen Vereins in Wien 14: 239–288.

Steyermark, J.A., 1974. Relación florística entre la Cordillera de la Costa y la zona de Guyana y Amazonas. Acta Botánica Venezuelaica 9, 245–252.

Steyermark, J.A., 1979. Plant Refuge and Dispersal centres in Venezuela: Their Relict and Endemic Element. In: Larsen, K., Holm-Nielsen, L.B. (Eds.), Tropical Botany. Academic Press, London, pp. 185–221.

Stuart, L.C. 1963. A checklist of the herpetofauna of Guatemala. Misc. Publ. Mus. Zool., Univ. Michigan, 122, 1-150.

Swofford D. L, Olsen G. J, Waddell P J, Hillis D M. 1996. Phylogenetic inference. *In* Molecular systematics,D. M. Hillis, C. Moritz and B. K. Mable (eds.). 2nd edition, Sinauer, Sunderland. p. 407-514.

Taboada, A., & Rivera, L. 2000. Geodynamics of the northern Andes', *19*(5), 787–813.

Taboada, C., Grant, T., Lynch, J.D. & Faivovich, J. 2013. New morphological synapomorphies for the new world direct-developing frogs (Amphibia, Anura, Terrarana). Herpetologica, 69, 342–357.

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0.

Tihen, J. A. 1962. A review of New World fossil bufonids. American Midland Naturalist, 68(1), pp.1-50.

Torres-Carvajal, O. 2007. Phylogeny and biogeography of a arge radiation of Andean lizards (Iguania, Stenocercus). Zoologica Scripta, 36, 311–326.

Thomé MTC, Zamudio KR, Haddad CFB, Alexandrino J. 2014. Barriers, rather than refugia, underlie the origin of diversity in toads endemic to the Brazilian Atlantic Forest. Molecular Ecology 23(24): 6152-6164. http://dx.doi.org/10.1111/mec.12986

Trueb, L. 1970. Evolutionary relationships of casque-headed tree frogs with co-ossified skulls (family Hylidae). Univ. Kansas Publ. Mus. Nat. Hist., 18, pp.547–716.

Trueb, L. 1973. Bones, frogs, and evolution. In: J. L. Vial, ed. Evolutionary Biology of the Anurans: Contemporary Research on Major Problems. University of Missouri Press [Columbia], pp.65-132.

UAESPNN, 2005. Plan de Manejo Parque Nacional Natural Sierra Nevada de Santa

Uba, C.E., Heubeck, C., Hulka, C., 2006. Evolution of the late Cenozoic Chaco foreland basin, southern Bolivia. Basin Res. 18, 145–170.

Van der Hammen, T., Werner, J. H., & van Dommelen, H. (1973). Palynological record of the upheaval of the Northern Andes: A study of the pliocene and lower quaternary of the Colombian Eastern Cordillera and the early evolution of its high-Andean biota. *Review of Palaeobotany and Palynology*, *16*(1-2), 1–122. http://doi.org/10.1016/0034-6667(73)90031-6

Varón, A., L. S. Vinh, W. C. Wheeler. 2010. POY version 4: phylogenetic analysis using dynamic homologies. Cladistics, 26:72-85.

Varón, A., & Wheeler, W. C. 2012. The tree alignment problem. *BMC Bioinformatics*, *13*, 293. Retrieved from

Weigend, M. 2004. Additional observations on the biogeography of the Amotape-Huancabamba zone in Northern Peru : Defining the South-Eastern limits. *Rev. Peru. Biol.*, *11*(2), 127–134. http://doi.org/10.1663/0006-8101(2002)068

Weir, J. T., & Price, M.. 2011. Andean uplift promotes lowland speciation through vicariance and dispersal in Dendrocincla woodcreepers. *Molecular Ecology*, *20*(21), 4550–4563. http://doi.org/10.1111/j.1365-294X.2011.05294.x

Wesselingh, F.P., Räsänen, M.E., Irion, G., Vonhof, H.B., Kaandorp, R., Renema, W., Romero Pittman, L. & Gingras, M. 2002. Lake Pebas: A palaeoecological reconstruction of a Miocene, long-lived lake complex in western Amazonia. *Cainozoic Res.* 1: 35–81.

Wesselingh, F.P. & Salo, J.A. 2006. Miocene perspective on the evolution of the Amazonian biota. *Scripta Geologica*, **133**: 439-458.

Wheeler, W. C. 1996. Optimization Alignment: the End of Multiple Sequence Alignment in Phylogenetics? *Cladistics*, *12*(1), 1–9. Retrieved from ttp://doi.wiley.com/10.1006/clad.1996.0001

Wheeler, W.C. 1998. Alignment characters, dynamic programing and heuristic solutions. In: R.DeSalle, and B. Schierwater, ed, Molecular approaches to ecology and evolution, 2nd ed, pp.243–251.

Wheeler, W.C. 2012. Systematics, A Course of Lectures. Wiley-Blackwell, Hoboken, New Jersey, 446 pp.

Willis, E. O. 1992. Zoogeographical Origins of Eastern Brazilian. *Ornitologia Neotropical*, *3*(1), 1–15. http://doi.org/10.1002/mmnd.4800250112

Willmott, K. R., Hall, J. P. W., & Lamas, G. 2001. Systematics of \emph{{Hypanartia}} ({Lepidoptera}: {Nymphalidae}: {Nymphalinae}), with a test for geographical speciation mechanism in the {Andes}. *Systematic Entomology*, *26*, 369–399.

Wüster W, Ferguson JE, Quijada-Mascareñas . 2005. Tracing an invasion: landbridges, refugia, and the phylogeographyof the Neotropical rattlesnake (Serpentes: Viperidae:*Crotalusdurissus*). *Molecular Ecology*14(4), 1095–1108.

Zamudio, K.R. & Green, H.W. 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics, and conservation. *Biological Journal of the Linnaean Society*, **62**: 421-442.

Zwickl, D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation, The University of Texas at Austin, 115 pp.