

**PHYLOGENETIC ANALYSIS WITHIN THE
PRISTIMANTIS UNISTRIGATUS (ANURA,
CRAUGASTORIDAE) GROUP BASED ON
MORPHOLOGICAL CHARACTERS**

**Análisis filogenético en el grupo *Pristimantis unistrigatus* (Anura,
Craugastoridae) basado en caracteres morfológicos**

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ABSTRACT

We present a phylogenetic analysis within the *Pristimantis unistrigatus* group (Anura, Craugastoridae) of Colombia. Characters from the superficial muscles of the hands and feet as well as external characters were taken for analysis. Most of the muscle characters were observed directly, and some were taken from the literature. Similarly, the external ones were taken mostly from the original descriptions and others from the literature as well. Two matrices were constructed, as the species belonging to this group have changed in recent years with respect to the initially proposed when the group was defined. The results lead us to conclude that the group is not monophyletic, although there are some relationships that are worth to survey because they are kept in the very last cladograms obtained for both proposals. It is suggested that these last relationships should be explored in particular, and the overall group in general, increasing the number of characters and taxa that belong to *P. unistrigatus*. An open question we left is whether actually is worth to keep these informal taxonomic hierarchy called group within the genera of anurans.

Key words. Neotropical frogs, Cordillera Oriental, superficial muscles, *Pristimantis*.

RESUMEN

Se presenta un análisis filogenético del grupo *Prsitimantis unistrigatus* (Anura, Craugastoridae) de Colombia. Para esto se tomaron caracteres de la musculatura superficial de manos y pies y caracteres externos. La mayoría de los caracteres musculares fueron observados directamente, y algunos fueron tomados de la literatura. Igualmente, los externos fueron tomados en su mayoría de las descripciones originales,

y otros tomados de la literatura también. Se construyeron dos matrices, pues las especies que pertenecen al grupo han cambiado en los últimos años con respecto a lo propuesto inicialmente cuando el grupo fue definido. Los resultados nos llevan a concluir que el grupo no es monofilético, aunque existen algunas relaciones que vale la pena explorar, pues se mantienen en los cladogramas definitivos obtenidos para ambas propuestas. Se sugiere que deben explorarse más estas relaciones en particular, y la del grupo en general, aumentando los caracteres y el número de taxones que pertenecen a *P. unistrigatus*. Igualmente se deja como interrogante si en realidad vale la pena mantener estos grupos informales dentro de los géneros de anuros.

Palabras clave. Ranas neotropicales, Cordillera Oriental, músculos superficiales, *Pristimantis*.

INTRODUCTION

Frost *et al.* (2006) doubted the existence of the genus *Eleutherodactylus* Duméril and Bibron, 1841 as monophyletic, and suggested that it should be divided into many monophyletic genera. Heinicke *et al.* (2008) divided the former genus *Eleutherodactylus* plus the genus *Brachycephalus* Fitzinger, 1826 in three Clades: the South American Clade, the Caribbean Clade and the Middle American Clade. The *Eleutherodactylus unistrigatus* group was included in the South American Clade using the available name *Pristimantis* Jiménez de la Espada, 1870. This group is one of the richest *Pristimantis* groups, with members occurring in the Andes and the Amazonian basin, ranging from Bolivia to Colombia (Hedges *et al.* 2008, Padiál *et al.* 2009).

In the literature related to the diagnosis of the *unistrigatus* group species of the Cordillera Oriental of Colombia, potential kinship and characteristics in common are referred (Duellman & Simmons 1977, Lynch 1973, 1978, 1981, 1983, 1984, 1994a, 1994b, 1998, 2003, Lynch & Duellman 1980, Lynch & Duellman 1997, Lynch & Suarez 2002, Pyburn & Lynch 1981). However, there have been no formal relationship hypotheses supported by cladograms.

It is perhaps for this *Pristimantis* group that were proposed the highest number of

hypotheses of relationships in Colombia, because it is the richest in species number in this country. From the perspective of phylogenetic systematics of the genus *Pristimantis*, the work that has a growing number of Colombian species for the *unistrigatus* group (*sensu* Lynch & Duellman 1997) of the Cordillera Oriental of Colombia has been that of Galindo (2004), based on external characters. However, the papers of Duellman & Simmons (1977), Lynch (1973, 1975a, 1975b, 1978, 1981, 1983, 1984, 1994a, 1994b, 1998, 2003), Lynch & Duellman (1980), Lynch & Suárez (2002) and Pyburn & Lynch (1981) have revealed potential relationships for species of the *unistrigatus* group, some with formal cladograms, but the majority without them. Lynch (1984) proposed a hypothesis of relationship for the *unistrigatus* group without including any of the species used here, but some belonging to the “assemblages” *pyrrhomerus* and *myersi*, suggesting these should be combined by a series of synapomorphies found in external characters. Finally, recently Padiál *et al.* (2009) considered that the *Pristimantis unistrigatus* group is not monophyletic.

Other groups, which have launched hypotheses of relationship, either with or without explicit cladograms are: *discoidalis* (Lynch 1989) (with explicit cladogram), using external and cranial characters, and *sulcatus* (Lynch 1997) (with explicit cladogram), finding for

these relationships nine characters from the skull. As this paper aims to use morphology as a fundamental element to establish kinship hypothesis, the discussion will not be based on specific morphological differences between what we found and what other authors have found, but between the relationships proposed here based on potential synapomorphies, and other hypotheses and characters used to construct them.

From morphology's point of view, the contributions are all innovative in the sense that no previous research has been dedicated solely to the study of muscles of hands and feet for the phylogenetic analysis of the *Pristimantis* genus. Similarly, although there are some previous descriptions about hand muscles of some species of the genus (e.g. Burton 1998a, Dunlap 1960), these were mainly for comparative purposes (Dunlap 1960, Salgar *et al.* 2009), or with the aim to find characters to establish hypotheses of relationships (Burton 1998a) but there was not a genus-specific analysis performed.

Previous studies in the literature, using both muscles of hands and forearms and feet to propose phylogenetic relationships in frogs are very rare; in fact, there is only one (Faivovich 2002), but in the genus *Scinax* (Hylidae). The most comprehensive descriptive work in this regard have been those of Gaup (1886), Dunlap (1960), Andersen (1978) and, more recently, Burton (1996, 1998a,b, 2001, 2004). Most of them propose (tacitly or explicitly) some assumptions about the potential systematic value of characters taken from hands and feet in anuran amphibians, without reaching cladistic analysis themselves. Only in one of his most recent work, Burton (2004) developed a cladistic analysis using only the muscles of the feet in the family Hylidae. The only existing descriptions of this musculature in Colombian species of frogs has been conducted by Salgar (2003) and Salgar *et al.* (2009). Although these last two

analyses are brief, for the first time, possible synapomorphies for the genus *Pristimantis* (intrageneric relationships) based on the muscles of hands and feet were revealed. Lynch (1984) proposed some characters but for relationships between species groups, based on osteological characters and some of the muscles (but not hands and feet).

Hedges *et al.* (2008) redefined the *Pristimantis unistrigatus* group changing the species included to it, assigning 193 species to this group, changing some of the species previously identified into the group by Lynch (1976a, Lynch & Duellman 1997, Duellman & Pramuk 1999), and by Lynch & Duellman (1997) and Acosta (2000) for Colombia.

Taking into account that there are two different classifications for the *Pristimantis unistrigatus* group, our aim is to compare our results in using morphological characters from hand and foot muscles in both proposals, because there are not enough consensus about which is the best classification. It is for this reason that both the number of species and characters in both analyses differs (see results). The only classification that include all species of our study is that by Lynch & Duellman (1997). The number of species in classification by Hedges *et al.* (2008) is lower, because they do not consider some of the species by Lynch & Duellman (1997) as belonging to the *P. unistrigatus* group. Owing to this account, the number of characters also changed. In comparing our results with those by Heinicke *et al.* (2008), and Hedges *et al.* (2008), we can conclude that they agree that *P. unistrigatus* group is not monophyletic.

MATERIALS AND METHODS

A total of 42 adult specimens (Table 1) representing 17 species of *Pristimantis* of the Cordillera Oriental of Colombia and one of *Craugastor* were dissected. Specimens belong to the Museo de Historia

Natural (MUJ) of the Pontificia Universidad Javeriana in Bogotá, Colombia, and to the Instituto de Ciencias Naturales (ICN) of the Universidad Nacional de Colombia in Bogotá. Taking into account the species belonging to the *unistrigatus* group *sensu* Hedges *et al.* (2008), we decided to compare results considering the former classification made by Lynch & Duellman (1997). We chose the following two ingroups: *sensu* Lynch & Duellman (1997) (LD): *Pristimantis anolirex* (Lynch, 1983): ICN 33522, 15444; *P. bacchus* (Lynch, 1984): ICN 33164, 33170, 34232; *P. bicolor* (Rueda-Almonacid and Lynch, 1983): ICN 14474, 26321; *P. bogotensis* (Peters, 1863): MUJ 052, 465, 481, 687; *P. elegans* (Peters, 1863): MUJ 036; *P. frater* (Werner, 1899): ICN 40579, 49911; *P. jorgevelosai* (Lynch, 1994): ICN 15299, 15318; *P. lynchi* (Duellman and Simmons, 1977): MUJ 1480, 1538, 1507, 2036; ICN 01932, 01937; *P. merostictus* (Lynch, 1984): ICN 34233, 34235; *P. miyatai* (Lynch, 1984): ICN 50062, 50065, 50069; *P. nervicus* (Lynch, 1994): MUJ 023, 1176; *P. nicefori* (Cochran and Goin, 1970): ICN 20994, 21119; *P. prolixodiscus* (Lynch, 1978): ICN 10104, 10107; *P. pugnax* (Lynch, 1973): ICN 22981, 23187; *P. spilogaster* (Lynch, 1984): ICN 12445, 12446; *P. taeniatus* (Boulenger, 1912): MUJ 2452, 2420; *P. tamsitti* (Cochran and Goin, 1970): ICN 22949, 22951, 23638; *Sensu* Hedges *et al.* (2008) (HEA): *P. anolirex* (Lynch, 1983), *P. bacchus* (Lynch, 1984), *P. bogotensis* (Peters, 1863), *P. elegans* (Peters, 1863), *P. lynchi* (Duellman and Simmons, 1977), *P. merostictus* (Lynch, 1984), *P. nervicus* (Lynch, 1994), *P. nicefori* (Cochran and Goin, 1970), *P. pugnax* (Lynch, 1973), *P. spilogaster* (Lynch, 1984), *P. taeniatus* (Boulenger, 1912), *P. tamsitti* (Cochran and Goin, 1970).

Outgroups: *Craugastor raniformis*: MUJ 2871, 2689 for the LD matrix, and *P. frater* for the HEA matrix (Tables 2 and 3).

Table 1. List of species. M: Male. F: Female.

Specimen	Sex	Species
MUJ 36	M	<i>P. elegans</i>
MUJ 052	F	<i>P. bogotensis</i>
MUJ 465	M	<i>P. bogotensis</i>
MUJ 481	M	<i>P. bogotensis</i>
MUJ 687	F	<i>P. bogotensis</i>
MUJ 23	F	<i>P. nervicus</i>
MUJ 1176	F	<i>P. nervicus</i>
MUJ 1507	F	<i>P. lynchi</i>
MUJ 2036	F	<i>P. lynchi</i>
MUJ 2420	M	<i>P. taeniatus</i>
MUJ 2452	F	<i>P. taeniatus</i>
MUJ 1480	F	<i>P. lynchi</i>
MUJ 1538	M	<i>P. lynchi</i>
ICN 33522	F	<i>P. anolirex</i>
ICN 15444	F	<i>P. anolirex</i>
ICN 26321	M	<i>P. bicolor</i>
ICN 50062	F	<i>P. miyatai</i>
ICN 50065	M	<i>P. miyatai</i>
ICN 50069	F	<i>P. miyatai</i>
ICN 33170	F	<i>P. bacchus</i>
ICN 34232	M	<i>P. bacchus</i>
ICN 33164	M	<i>P. bacchus</i>
ICN 12445	M	<i>P. spilogaster</i>
ICN 12446	F	<i>P. spilogaster</i>
ICN 1932	F	<i>P. lynchi</i>
ICN 1937	F	<i>P. lynchi</i>
ICN 14474	F	<i>P. bicolor</i>
ICN 40579	M	<i>P. frater</i>
ICN 49911	M	<i>P. frater</i>
ICN 15299	M	<i>P. jorgevelosai</i>
ICN 15318	M	<i>P. jorgevelosai</i>
ICN 10107	M	<i>P. prolixodiscus</i>
ICN 10104	F	<i>P. prolixodiscus</i>
ICN 22981	F	<i>P. pugnax</i>
ICN 23187	F	<i>P. pugnax</i>
ICN 21119	M	<i>P. nicefori</i>
ICN 20994	F	<i>P. nicefori</i>
ICN 22949	F	<i>P. tamsiti</i>
ICN 22951	F	<i>P. tamsiti</i>
ICN 23638	F	<i>P. tamsiti</i>
ICN 34233	F	<i>P. merostictus</i>
ICN 34235	F	<i>P. merostictus</i>

The new classification proposed by Hedges *et al.* (2008) for some of the species formerly assigned to *unistrigatus* group is as follows: *P. frater*: *frater* group; *P. bicolor*: species series *P. (Hypodiction) ridens*; *P. miyatai*: *P. frater* group; *P. jorgevelosai*: species series *P. (Hypodiction) ridens*; and *P. prolixodiscus*: *lacrimosus* group.

Muscles

We observed about 90 small hand and feet muscles, both superficial and deep, of which about 50 superficial were chosen. For choosing the characters and states, we did comparisons between muscles from ten species of *Pristimantis* used for the study with respect to different aspects that are related below. Dissections were done in hands, feet, and legs and forearms in order to examine those muscles associated morphologically with the hands and feet. We used a dissecting microscope and a reversible Weigert's iodine solution that stains muscles (Bock & Shear 1972) to see the direction of the fibers, obtaining a clear differentiation of muscles and tendons, including the origins and insertions. For all specimens both right and left hands and feet superficial muscles were dissected and examined, following the protocols proposed by Raikow *et al.* (1990). For each muscle we identified: a. origin; b. insertion; c. spatial arrangement with respect to the hand and foot, and d. spatial relationship with adjacent muscles. Although we explored mainly superficial musculature, we also dissected some deep muscles considering that we took into account characters proposed by Burton (1998a, 2004). The nomenclature was taken from that proposed by Dunlap (1960), Andersen (1978), Burton (1998a, 2004), and Salgar *et al.* (2009). We used for most cases, at least one male and one female of each species to the extent that the number of specimens of the collection allowed it, to determine the existence of sexual dimorphism. Finally, we drew pictures of characters considered as potential synapomorphies using photographs and the Adobe Illustrator software. For the accuracy of the origin and insertion of muscles, we made use of clearing and staining skeletons of *P. bogotensis*.

External characters

These were taken from taxonomic descriptions after Cochran & Goin (1970), Lynch (1983), Lynch (1984), Rueda & Lynch (1983), Dunn (1944), Hoyos (1991), Pyburn & Lynch (1991), Lynch (1994), Duellman & Simmons (1997), Lynch (1998), Lynch (1978), and Lynch & Duellman (1980), and after our own observations.

Phylogenetic analysis

For the phylogenetic analysis, we compared each result taking into account two matrices (see below). We did heuristic searches in TNT, performing TBR branch swapping on 100 addition replicates. Matrices were running considering characters with the same weight. We did not find many intraspecific (polymorphic) variations or many multistate characters, however we use all of those we found for the phylogenetic analyses. Polymorphic characters were coded according to Faivovich (2002) as follows: A=0/1; B=0/2, and C=1/2, and multistate characters were all considered nonadditive and unordered. We did not get whether retention (RI) or consistency indices (CI), given that we are comparing groups of different sizes (e.g. Conrad 2008), so the value for the CI will be greater in the HEA (*Sensu* Hedges *et al.*, 2008) group than in the LD (*sensu* Lynch & Duellman, 1997) group because we considered less species in the first group than in the second one, and the RI will be less in the HEA for the same reason.

We choose one outgroup species for each analysis, following recommendation by Nixon & Carpenter (1993: 419) about the fact that "...a third pervasive myth, the notion that more than one outgroup is somehow required for correct polarity determination", and taking into account that "Outgroups and polarity are the same problem" (Nixon & Carpenter 1993: 420). We only show the unambiguous synapomorphies.

Table 2. Data matrix employed in the cladistic analysis of the LD *P. unistrigatus* species group.

<i>Craugastor raniformis</i>	0000	0010	0000	1010	0000	0100	0000	0111	10101100111101101100111100001110000111010110000
<i>Pristimantis taeniatus</i>	2000	0001	0000	0010	0000	0000	0011	1111	000111001001000011020110101000000000100
<i>Pristimantis tamsitti</i>	2000	0001	0010	1010	1010	1100	0110	0101	A2010A0120010101A000110100111001001001
<i>Pristimantis pugnax</i>	1000	0001	0010	0010	0000	1001	00C1	0111	00010001200001010001100011011011000010000
<i>Pristimantis bacchus</i>	1010	1001	0000	0000	0000	1000	0010	0110	110101120010101000011101010001010100
<i>Pristimantis miyatai</i>	2010	1001	1100	1010	0010	1000	0010	1110	00010010000001002011010001000000100
<i>Pristimantis merostictus</i>	1000	1001	0100	1010	0000	0000	0010	0100	100101000001010010110102010000000100
<i>Pristimantis spilogaster</i>	1010	1111	0100	1011	0100	0001	0011	0111	1011010120010101001011000101000000100
<i>Pristimantis anolirex</i>	2001	1001	0010	0010	0000	0001	0011	0111	A00A11010001010A102A11000100000000100
<i>Pristimantis jorgevelosai</i>	2001	1001	0010	0010	0100	0001	1011	0110	1001010120010101100011010101001000000100
<i>Pristimantis nicefroi</i>	2001	AA10	B010	1000	0000	0000	0010	1111	10110A0120010102010020110000010111010110
<i>Pristimantis bicolor</i>	2000	1111	0010	1011	0000	0111	0111	0111	100111100001000011000201010101010000010100
<i>Pristimantis frater</i>	20A1	1101	0001	0100	0000	0001	0010	0011	0001010120000011020110101011000000100
<i>Pristimantis prolixodiscus</i>	2101	1101	0000	0100	0010	0111	101A	0111	10A11000000001011020000001210000110100
<i>Pristimantis bogotensis</i>	2000	0001	0000	0010	0001	1000	0010	0110	0011110100100000000011010001000100000100
<i>Pristimantis elegans</i>	2000	0001	0000	0010	0001	0000	0010	0111	000110110000111002111010011000100000100
<i>Pristimantis nervicus</i>	2000	000A	0000	0010	0001	0000	0010	1111	100101101001011110201100012100001010100
<i>Pristimantis lynchi</i>	2000	0001	0100	0010	0001	0100	0010	0111	00011100100100001A021110101010000011000

Table 3. Data matrix employed in the cladistic analysis of the HEA *P. unistrigatus* species group.

<i>Pristimantis frater</i>	1A11101000101000000011000011000101012000001120010101100000100
<i>Pristimantis taeniatus</i>	10000010000010000000011110001100101001020010100000000100
<i>Pristimantis tamsitti</i>	10000010010101101101000101A2010A01201100100010011100101001
<i>Pristimantis pugnax</i>	00000010010001000010A1011100010001200101100011011100010000
<i>Pristimantis bacchus</i>	0101001000000000010000001101101112011010000110101000110100
<i>Pristimantis merostictus</i>	00010010101000000000100101010001010010201000000100
<i>Pristimantis spilogaster</i>	010111010010110100000111011010120110100000101000000100
<i>Pristimantis anolirex</i>	10110010010001000001001111A00A110100110A12A000100000000100
<i>Pristimantis nicefroi</i>	101AA10B010100000000001111010A01201101020000001011110110
<i>Pristimantis bogotensis</i>	10000010000001000110000001100011110101000000010001000100001
<i>Pristimantis elegans</i>	10000010000001001000000110001101100110210100100010000100
<i>Pristimantis nervicus</i>	100000A0000001000100000011110010110101111200001210000110100
<i>Pristimantis lynchi</i>	1000001010000100010100000011100011100101001A210101010000011000

Results

We scored 81 characters, from which 64 are muscle characters (44 from the manus and 20 from the pes), and 17 external characters. Four manus characters were taken from Burton (1998a), and five pes characters from Burton (2004). Based on this matrix, we eliminated those characters according to changes in the taxa for each matrix as we describe below.

Phylogenetic analysis

LD *P. unistrigatus* group

By eliminating those characters with only one character state present in the outgroup and ingroup, we took 70 from the 81 characters (Appendix 1). The cladistic analysis resulted in one fully resolved most-parsimonious tree (Fig. 1) with a length of 202. We found character states 62(0) (First finger shorter than second) and 23(1) (The origin of the *m. adductor policis* is not covered by the *aponeurosis palmaris*) (Fig. 2) as the only two unambiguous putative synapomorphies. The first one included almost all species

leaving away *P. nicefori*, and the second one is a character shared by *P. prolixodiscus* and *P. bicolor*.

HEA *P. unistrigatus* group (Appendix 2)

We took 60 characters for the analysis (Appendix 2). The cladistic analysis resulted in 20 most-parsimonious trees of 139 steps. The strict consensus tree (Fig. 3) shows a node 14 not resolved, consisting of taxa *P. lynchi*, *P. nervicus*, *P. elegans*, *P. bogotensis*, *P. nicefori*, *P. anolirex*, *P. bacchus*, and *P. taeniatus*. The only unambiguous putative synapomorphy (found in all trees) is the character state 44(1) (Origin of the *tendo superficialis hallucis* from the *aponeurosis plantaris* including some fibers of the *m. lumbricalis brevis hallucis*) (Fig. 4) relating *P. spilogaster* and *P. merostictus*.

DISCUSSION

As shown in the cladograms obtained for both *unistrigatus* groups, the LD (Fig. 1) group is more resolved than the strict consensus of the HEA (Fig. 3), but there are no synapomorphies

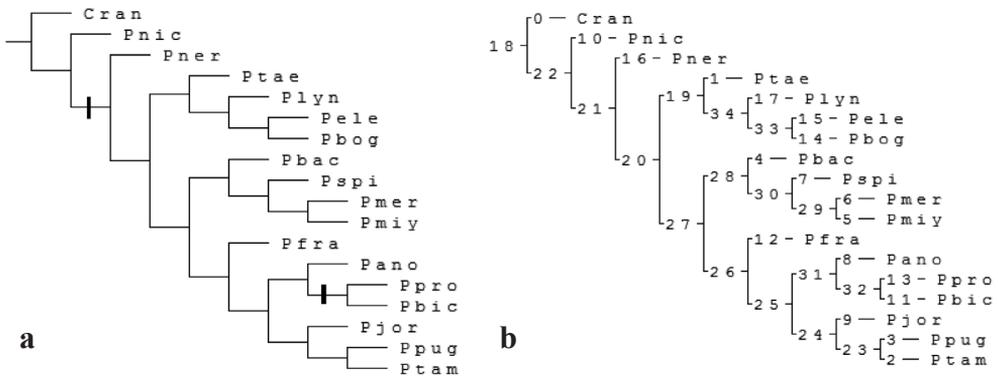


Figure 1. The only most-parsimonious cladogram of LD *P. unistrigatus* group. a. Tree without showing nodes numbers, and showing the character states 23 (1) and 62 (0) as unambiguous synapomorphies. b. Tree showing nodes numbers.

Abbreviations: Cran: *Craugastor raniformis*, Pano: *Pristimantis anolirex*, Pbac: *P. bacchus*, Pbic: *P. bicolor*, Pbog: *P. bogotensis*, Pele: *P. elegans*, Pfra: *P. frater*, Pjor: *P. jorgevelosai*, Plyn: *P. lynchi*, Pmer: *P. merostictus*, Pmiy: *P. miyatai*, Pner: *P. nervicus*, Pnic: *P. nicefori*, Ppro: *P. prolixodiscus*, Ppug: *P. pugnax*, Pspi: *P. spilogaster*, Ptae: *P. taeniatus*, Ptam: *P. tamsitti*.

that support it, except partially for nodes 21 and 32 in LD, and exclusively for node 16 in HEA, this means that all topologies are weakly supported by synapomorphies. In node 22 of DL no synapomorphies were found, whereas for the other nodes, potential synapomorphies are actually reversals or convergences.

Taking into account the results and species considered by HEA and LD, we could say that the assigned HEA *unistrigatus* group is weaker because the relationships are less resolved, even if the number of steps is smaller (it has a smaller number of taxa). On the other hand, even with a larger number of species involved,

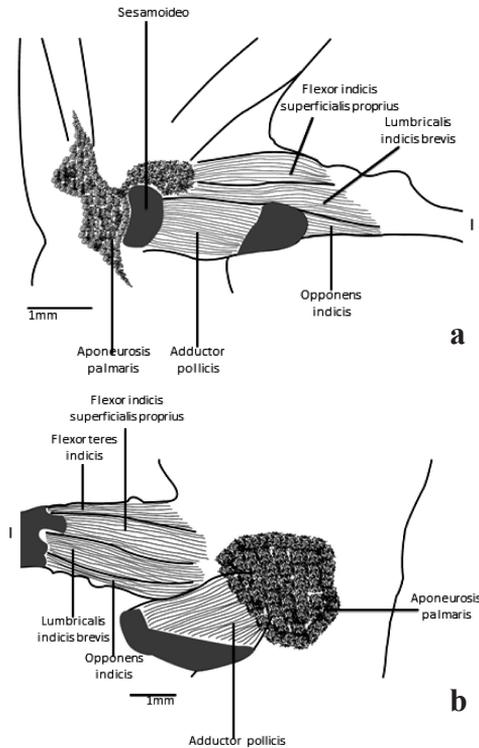


Figure 2. a. Ventral view of the manus showing the two conditions of the character 23 in the LD matrix: a. origin of the *m. adductor pollicis* is not covered by the *aponeurosis palmaris* (23-1). *P. bicolor* (ICN 26321). b. Origin of the *m. adductor pollicis* is covered by the *aponeurosis palmaris*. *C. raniformis* (23-0). MUJ 2871

the proposed LD *unistrigatus* group can be considered stronger if we take the fact that we have got a single tree completely resolved;

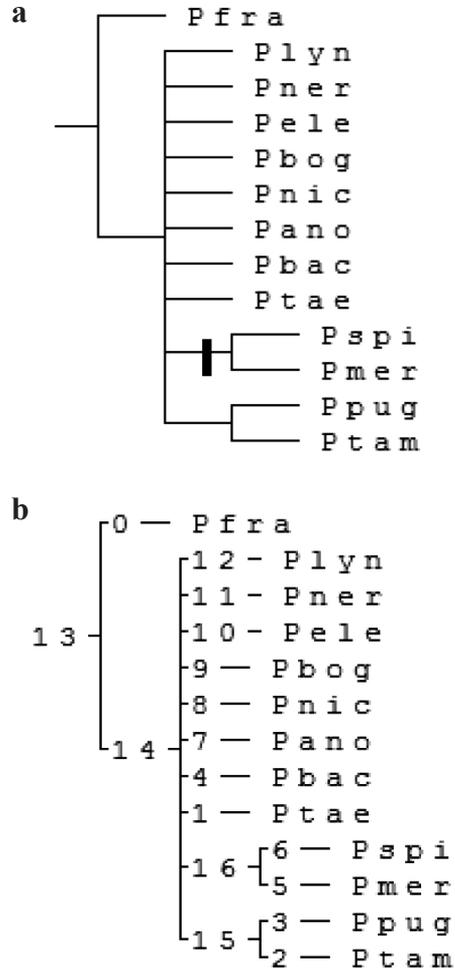


Figure 3. Strict consensus of the 20 most-parsimonious cladograms of HEA *P. unistrigatus* group. a. Tree without showing nodes numbers, and showing the character state 44 (1) as an unambiguous synapomorphy b. Tree showing nodes numbers.

Abbreviations: Cran: *Craugastor raniformis*, Pano: *Pristimantis anolirex*, Pbac: *P. bachus*, Pbic: *P. bicolor*, Pbog: *P. bogotensis*, Pele: *P. elegans*, Pfra: *P. frater*, Pjor: *P. jorgevelosai*, Plyn: *P. lynchi*, Pmer: *P. merostictus*, Pmiy: *P. miyatai*, Pner: *P. nervicus*, Pnic: *P. nicefori*, Ppro: *P. prolixodiscus*, Ppug: *P. pugnax*, Pspi: *P. spilogaster*, Ptae: *P. taeniatus*, Ptam: *P. tamsitti*.

this does not mean that we are clear about the putative synapomorphies involved, as we saw above. It is remarkable that the relationships between *P. pugnax* and *P. tamsitti*, and between *P. spilogaster* and *P. merostictus* are retained in both cladograms, meaning that these species maybe considered sisters, but without finding putative synapomorphies to support the relationship between *P. pugnax* and *P. tamsitti*.

Limb muscles and phylogeny

All characters and character states obtained were chosen bearing in mind character distribution and conditions deemed to be worth for the analysis. Burton's (1998a, 2004) characters and character states were used just as he defined them, but in some cases we believed that some had to be redefined due to the fact that they were not clearly named. Then, we think that most of these characters are new and very useful for future analyses in frog morphology and phylogeny studies. It is fascinating how many characters have found on the hands and feet, although only the superficial muscles have been observed. More characters were found in the hands

(44, including four of Burton) than in the feet (21, including six of Burton). Similarly, after making a clearance based on external descriptions and original observations, we obtained 26 characters. Faivovich (2002) used 14 characters from the manus and seven from the pes, from which he found two synapomorphies for *Scinax* Wagler, 1830, both from the manus. However, although we did not find any useful characters taken from hand and foot musculature, this does not mean that these characters should be dismissed from phylogenetic studies in frogs. Burton (1998a) found some hand muscle character conditions useful to support intrafamilial relationships in Leptodactylids. Likewise, Burton (1998b) found a hand muscle character condition within Ranidae Rafinesque, 1814, stressing that superficial muscles can vary more than deep ones, but that these muscle can contribute to get frog relationships. It is apparently historically clear that superficial hand muscles may play a role more important to resolve frog relationships than superficial feet muscles, although we found muscles of both that could be putative synapomorphies.

External characters

Phylogenetic analysis including external characters is rare in frogs, although identification of most of the species groups and all species have been based on these characters. The results show that characteristics of the fingers are still important, and can be used in establishing kinship, but were obviously not essential to support the monophyly of the *unistrigatus* group. Apparently, the other external characters are not useful because, in any case, it was established that they could participate as potential synapomorphies. Maybe we should pay attention to the condition of tympanic annulus and to the dorsal view of the snout because for the two analyses that show that these characters appear as potential synapomorphies (nodes 21 and 23 respectively in LD group, and node

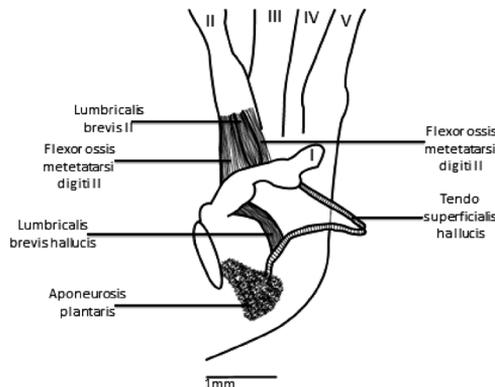


Figure 4. Ventral view of the pes showing the apomorphic state of the character state 44 (1). Origin of the *tendo superficialis hallucis* from the *aponeurosis plantaris* including some fibers of the *m. lumbricalis brevis hallucis*. *P. mersotictus*. ICN 34235

15 in HEA group), although actually they are homoplasies.

To compare our results with those by Heinicke *et al.* (2008) and Hedges *et al.* (2008) is difficult, because most of the species included in our work were not used by these authors. However, our results agree in identifying the *unistrigatus* group as non monophyletic. Heinicke *et al.* (2008) only included six species belonging to the former *P. unistrigatus* group (Lynch, 1976; Lynch *et al.*, 1997). Taking into account this classification, their results showed that this group is monophyletic. However, based on Hedges *et al.* (2008) classification *P. ockendeni*, in the *P. unistrigatus* group by Heinicke *et al.* (2008), belongs to the *P. frater* group; in this sense, the group would become not monophyletic.

CONCLUSIONS

Although the muscles of hands and feet and external characters useful in taxonomy failed to identify the *Pristimantis unistrigatus* as monophyletic, this does not mean they are not useful for establishing relationships within the genus. It is possible that including more *unistrigatus* group species in the study, the situation changes, however, it seems also essential to include the deep muscles of the hands and feet and external characters as well. It is possible that other characters facilitate to define relationships among species within the genus. However, it is necessary to explore deeper the relationship *P. spilogaster*-*P. merostictus*, and *P. pugnax*-*P. tamsitti*, because we need whether to confirm or falsify if these clades actually are monophyletic. By detecting the difficulties that several researchers (e.g, Hedges *et al.* 2008; Padial *et al.* 2009) and our own study have found to discover the monophyly of the *P. unistrigatus* group and other ones in frogs, the question we pose is: Is it worthwhile to keep this informal taxonomic hierarchy called group in systematics of frogs?

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Appendix 1. List of characters and character states for the LD matrix.

Manus

- | | |
|--|---|
| <p>1. Number of insertions of the <i>m. extensor digitorum communis longus</i></p> <p>0 Four</p> <p>1 Three</p> <p>2 Two</p> <p>2. Number of origins of the <i>m. epicondylocubitalis</i></p> <p>0 Two, one slip in common with the <i>m. extensor carpi ulnaris</i> and the other slip free.</p> <p>1 One slip, not in common with the <i>m. extensor carpi ulnaris</i></p> <p>3. Relationship between the <i>m. epicondylocubitalis</i> and the <i>m. epitrochleocubitalis</i></p> <p>0 Bipennated</p> <p>1 Parallel</p> <p>4. Type of origin of the <i>m. epitrochleocubitalis</i></p> <p>0 Double</p> <p>1 Single</p> <p>5. Insertion of the <i>m. epitrochleocubitalis</i></p> <p>0 Not covered by the <i>m. palmaris brevis</i></p> <p>1 Covered by the <i>m. palmaris brevis</i></p> <p>6. Insertion of the <i>m. epicondylocubitalis</i> in relation with the <i>m. palmaris brevis</i></p> <p>0 It does not cover the <i>m. palmaris brevis</i></p> <p>1 It covers the <i>m. palmaris brevis</i></p> <p>7. Relationship between the <i>m. epitrochleocubitalis</i> and the external slip of the <i>m. palmaris longus</i></p> | <p>0 The <i>m. palmaris longus</i> is partially covered by the <i>m. palmaris longus</i></p> <p>1 The <i>m. palmaris longus</i> is not covered by the <i>m. palmaris longus</i></p> <p>8. Relationship between the <i>m. epitrochleocubitalis</i> and the <i>m. epicondylocubitalis</i></p> <p>0 The fibers fuse partially on the external surface of the radioulna</p> <p>1 The fibers completely fuse on the external surface of the radioulna</p> <p>9. Relationship between the internal slip of the <i>m. extensor digitorum communis longus</i> and the <i>m. extensor carpi radialis</i></p> <p>0 Proximally partially fused</p> <p>1 Completely fused</p> <p>2 Not fused</p> <p>10. Relationship between the <i>m. extensor brevis superficialis Digiti V</i> and the <i>m. abductor brevis Digiti V</i></p> <p>0 They are partially fused</p> <p>1 They are not fused</p> <p>11. Number of insertion of the <i>m. extensor brevis superficialis Digiti V</i></p> <p>0 One</p> <p>1 Two</p> <p>12. Origin of the <i>m. abductor brevis Digiti V</i></p> <p>0 From the distal carpal 5-4-3</p> |
|--|---|

Phylogenetic analysis within the *Pristimantis unistrigatus*

- 1 From the ulnare
13. Presence of the *m. abductor ulnocarpalis*
 0 Present
 1 Absent
14. Origin of the *m. palmaris longus*
 0 By two slips
 1 By one slip
15. Insertion of the *m. palmaris longus*
 0 Simple
 1 Double
16. Connection between the tendons of the *m. palmaris longus* and the flexor tendons
 0 Single tendon that divides to become *tendones superficiales*(TS) IV and V
 1 Two tendons that cross over before becoming TS IV and V
17. Relationship between the fibers of the *m. palmaris brevis* and the *m. palmaris longus*
 0 The fibers of the *m. palmaris brevis* are dorsal with respect to the tendons of insertion of the *m. palmaris longus*
 1 The *m. palmaris brevis* is between the two tendons of insertion of the *m. palmaris longus*
18. Relationship between the insertion of the *m. palmaris brevis* and the tendons of insertion of the *m. palmaris longus*
 0 *m. palmaris brevis* articulates with the tendons of insertion of the *m. palmaris longus*
 1 The *m. palmaris brevis* does not articulate with the *m. palmaris longus*
19. Relationship between the *m. palmaris longus* and the *m. flexor carpi ulnaris*
 0 They are partially fused
 1 They are not fused
20. Origin of the *m. adductor pollicis*
 0 From the ventral surface of the carpal 5-4-3
 1 From the internal surface of the distal carpal 5-4-3
21. Insertion of the *m. adductor pollicis* on the prepollex
 0 Adjacent to the insertion of the *m. abductor pollicis*
 1 Covers the insertion of the *m. abductor pollicis*
22. Relationship between the *m. adductor pollicis* and the *m. abductor pollicis*
 0 Both muscles are independent
 1 They are fused
23. Relationship between the origin of the *m. adductor pollicis* and the *aponeurosis palmaris*
 0 The origin is covered by the *aponeurosis palmaris*
 1 The origin is not covered by the *aponeurosis palmaris*
24. Condition of the proximal end of the *m. lumbricalis brevis indicis*
 0 Dorsal to the *m. adductor pollicis*
 1 Contiguous to the *m. adductor pollicis*
25. Nature of the origin of the tendon of the *m. lumbricalis brevis Digniti III*
 0 Short and wide with respect to the muscle
 1 Long and thin with respect to the muscle
26. Origin of the *m. lumbricalis brevis Digniti III* in relation with the *tendo superficialis & caput. profundum Digniti III*
 0 Dorsal
 1 Laterodorsal
27. Presence of fibers of the *tendo superficialis & caput. profundum Digniti III*
 0 Only on the external surface of the tendon
 1 On the external and internal surface of the tendon
 2 Only on the internal surface of the tendon
28. Nature of the *m. lumbricalis brevis Digniti IV*
 0 Two slips
 1 Only one slip
29. Position of the insertion tendons of the *m. lumbricalis longus Digniti IV* with respect to the *tendo superficialis aponeurosis palmaris prodigniti IV*
 0 Lateral to the *tendo superficialis aponeurosis palmaris prodigniti IV*
 1 The insertion is via the *tendo superficialis aponeurosis palmaris prodigniti IV*
30. Origin of the *m. lumbricalis longus Digniti V*
 0 From the *aponeurosis palmaris* and the TS V
 1 Only from the TS V
31. Number of insertions of *m. lumbricalis longus Digniti V*
 0 One
 1 Two
32. Number of slips that compose the *m. lumbricalis brevis Digniti V*
 0 One
 1 Two
- Pes
33. Type of insertion of the *m. extensor digitorum communis longus*
 0 Single
 1 Multiple
34. Number of insertions of the *m. extensor brevis superficialis hallucis*
 0 Two
 1 Three
 2 Single
35. Number of insertions of the *m. extensor brevis superficialis Digniti II*
 0 One
 1 Two
36. Relationship between the insertions of the *m. extensor brevis superficialis Digniti II* and the *m. extensor digitorum communis longus*
 0 Common insertion between *m. extensor brevis superficialis Digniti II* and the internal slip of *m. extensor digitorum communis longus*
 1 Independent insertions
37. Presence of the *m. extensor brevis medius hallucis*

- 0 Absent
1 Present
38. Presence of the *m. extensor brevis medius Digiti II*
0 Absent
1 Present
39. Number of insertion slips of the *m. extensor brevis superficialis Digiti III*
0 One
1 Two
40. Relationship between the insertions of the *m. extensor brevis superficialis Digiti III* and the *m. extensor digitorum communis longus*
0 Common insertion between *m. extensor brevis superficialis Digiti III* and any slip of *m. extensor digitorum communis longus*
1 Independent insertions
41. Origin of the *m. extensor brevis superficialis Digiti IV*
0 By two independent slips
1 By two proximally fused slips
2 By one slip
42. Presence of the *m. extensor brevis superficialis Digiti V*
0 Absent
1 Present
43. Origin of the *m. abductor prehallucis*
0 From the internal surface of the *aponeurosis plantaris*
1 From the distal internal surface of the tarsus
44. Presence of the *m. abductor brevis plantaris hallucis*
0 Absent
1 Present
45. Origin of the *m. lumbricalis brevis Digiti II*
0 From the *aponeurosis plantaris*
1 From the proximal end of the *tendo superficialis hallucis*
46. Type of origin of the *m. lumbricalis brevis Digiti III*
0 Tendinous
1 Fleshy
47. Relationship between the external slip of the *m. lumbricalis brevis Digiti IV* and the internal slip of *m. lumbricalis brevis Digiti V*
0 Fused only in the origin
1 Fused beyond its origin
- Burton characters
- Manus
48. Presence of the internal slip of the *m. lumbricalis brevis Digiti III*
0 Absent
1 Present
49. Number of slips of the *m. lumbricalis longus Digiti IV*
0 Two
1 Only one, the internal slip
50. Origin of the *m. extensor brevis superficialis Digiti V*

- 0 From both, the ulnare and distal carpals 5-4-3
1 From the ulnare

Pes

51. Origin of the *tendo superficialis hallucis*
0 From the *aponeurosis plantaris*
1 From the *aponeurosis plantaris* including some fibers of the *m. lumbricalis brevis hallucis*
2 From a muscle that originates from the distal tarsal 2-3
52. Origin of the *tendo superficialis Digiti II*
0 From the *aponeurosis plantaris*
1 It serves as the insertion tendon of the *m. transversus plantae distalis*
53. Origin of the *tendo superficialis Digiti III*
0 From the *m. flexor digiti brevis superficialis* only
1 From the *aponeurosis plantaris*
2 From a superficial tendon that emerges centrally on the plantar surface of the *aponeurosis plantaris*
54. Number of insertion tendons of the *m. lumbricalis logissimus Digiti IV*
0 Two
1 One

External characters

55. Skin on venter
0 Areolate
1 Smooth
56. Presence of dorsolateral folds
0 Present
1 Absent
57. Nature of tympanic anulus
0 Complete
1 Incomplete
58. Dorsal view of snout
0 Rounded
1 Subacuminate
2 Acuminate
59. Shape of snout in profile
0 Rounded
1 Truncated
2 Acute
60. Presence of cranial crests
0 Present
1 Absent
61. Width of the upper eyelid in relation with the inter-orbital distance
0 Narrower than the inter-orbital distance
1 As wide as the inter-orbital distance
62. Length of fingers I and II
0 First finger shorter than second
1 First and second finger equally or almost equally long
2 First finger longer than second
63. Presence of finger pads or discs
0 Present
1 Absent
64. Presence of skin variations on the ulnar region

Phylogenetic analysis within the *Pristimantis unistrigatus*

- | | |
|---|---|
| 0 Present | 1 Absent |
| 1 Absent | 68. Presence of webbed toes |
| 65. Presence of skin variations on the tarsals | 0 Present |
| 0 Present | 1 Absent |
| 1 Absent | 69. Presence of toe pads or discs |
| 66. Presence of skin variations on the heel | 0 Present |
| 0 Present | 1 Absent |
| 1 Absent | 70. Presence of lateral fringes on toes |
| 67. Presence of supernumerary plantar tubercles | 0 Present |
| 0 Present | 1 Absent |

Appendix 2. List of characters and character states for the HEA matrix.

Manus

- | | |
|---|--|
| 1. Number of insertions of the <i>m. extensor digitorum communis longus</i> | 11. Origin of the <i>m. abductor brevis</i> <i>Digiti V</i> |
| 0 Three | 0 From the distal carpal 5-4-3 |
| 1 Two | 1 From the ulnare |
| 2. Relationship between the <i>m. epicondylocubitalis</i> and the <i>m. epitrochleocubitalis</i> | 12. Presence of the <i>m. abductor ulnocarpalis</i> |
| 0 Bipennated | 0 Present |
| 1 Parallel | 1 Absent |
| 3. Type of origin of the <i>m. epitrochleocubitalis</i> | 13. Origin of the <i>m. palmaris longus</i> |
| 0 Double | 0 By two slips |
| 1 Single | 1 By one slip |
| 4. Insertion of the <i>m. epitrochleocubitalis</i> | 14. Insertion of the <i>m. palmaris longus</i> |
| 0 Not covered by the <i>m. palmaris brevis</i> | 0 Simple |
| 1 Covered by the <i>m. palmaris brevis</i> | 1 Double |
| 5. Insertion of the <i>m. epicondylocubitalis</i> in relation with the <i>m. palmaris longus</i> | 15. Connection between the tendons of the <i>m. palmaris longus</i> and the flexor tendons |
| 0 It does not cover the <i>m. palmaris brevis</i> | 0 Single tendon that divides to become <i>tendones superficiales</i> (TS) IV and V |
| 1 It covers the <i>m. palmaris brevis</i> . | 1 Two tendons that cross over before becoming TS IV and V |
| 6. Relationship between the <i>m. epitrochleocubitalis</i> and the external slip of the <i>m. palmaris longus</i> | 16. Relationship between the fibers of the <i>m. palmaris brevis</i> and the <i>m. palmaris longus</i> |
| 0 The <i>m. epitrochleocubitalis</i> is partially covered by the <i>m. palmaris longus</i> | 0 The fibers of the <i>m. palmaris brevis</i> are dorsal with respect to the tendons of insertion of the <i>m. palmaris longus</i> |
| 1 The <i>m. epitrochleocubitalis</i> is not covered by the <i>m. palmaris longus</i> . | 1 The <i>m. palmaris brevis</i> is between the two tendons of insertion of the <i>m. palmaris longus</i> |
| 7. Relationship between the <i>m. epitrochleocubitalis</i> and the <i>m. apicondylocubitalis</i> | 17. Relationship between the <i>m. palmaris longus</i> . and the <i>m. flexor carpi ulnaris</i> |
| 0 The fibers fuse partially on the external surface of the radioulna | 0 They are partially fused |
| 1 The fibers completely fuse on the external surface of the radioulna | 1 They are not fused |
| 8. Relationship between the internal slip of the <i>m. extensor digitorum communis longus</i> . and the <i>m. extensor carpi radialis</i> | 18. Origin of the <i>m. adductor pollicis</i> |
| 0 Proximally partially fused | 0 From the ventral surface of the carpal 5-4-3 |
| 1 Completely fused | 1 From the internal surface of the distal carpal 5-4-3 |
| 2 Not fused | 19. Insertion of the <i>m. adductor pollicis</i> . on the prepollex |
| 9. Relationship between the <i>m. extensor brevis superficialis</i> <i>Digiti V</i> and the <i>m. abductor brevis</i> <i>Digiti V</i> | 0 Adjacent to the insertion of the <i>m. abductor pollicis</i> |
| 0 They are partially fused | 1 Covers the insertion of the <i>m. abductor pollicis</i> |
| 1 They are not fused | 20. Relationship between the <i>m. adductor pollicis</i> and the <i>m. abductor pollicis</i> |
| 10. Number of insertion of the <i>m. extensor brevis superficialis</i> <i>Digiti V</i> | 0 Both muscles are independent |
| 0 One | 1 They are fused |
| 1 Two | 21. Condition of the proximal end of <i>m. lumbricalis brevis indicis</i> |
| | 0 Dorsal to the <i>m. adductor pollicis</i> |
| | 1 Contiguous to the <i>m. adductor pollicis</i> |

22. Origin of the *m. lumbricalis brevis Digiti III* in relation with the *m. tendo superficialis & caput profundum Digiti III*
 0 Dorsal
 1 Laterodorsal
23. Presence of fibers of the *tendo superficialis & caput. profundum Digiti III*
 0 On the external and internal surface of the tendon
 1 Only on the internal surface of the tendon
24. Nature of the *m. lumbricalis brevis Digiti IV*
 0 Two slips
 1 Only one slip
25. Position of the insertion tendons of the *m. lumbricalis longus Digiti IV* with respect to the *tendo superficialis aponeurosis palmaris prodigiti IV*
 0 Lateral to the *tendo superficialis aponeurosis palmaris prodigiti IV*
 1 The insertion is via the *tendo superficialis aponeurosis palmaris prodigiti IV*
26. Origin of the *m. lumbricalis longus Digiti V*
 0 From the *aponeurosis palmaris*. and TS V
 1 Only from TS V
27. Number of insertions of *m. lumbricalis longus Digiti V*
 0 One
 1 Two
28. Number of slips that compose the *m. lumbricalis brevis Digiti V*
 0 One
 1 Two
- Foot
29. Type of insertion of the *m. extensor digitorum communis longus*
 0 Single
 1 Multiple
30. Number of insertions of the *m. extensor brevis superficialis hallucis*
 0 Two
 1 Three
 2 Single
31. Number of insertions of the *m. extensor brevis superficialis Digiti II*
 0 One
 1 Two
32. Relationship between the insertions of the *m. extensor brevis superficialis Digiti II* and the *m. extensor digitorum communis longus*.
 0 Common insertion between *m. extensor brevis superficialis Digiti II* and the internal slip of the *m. extensor digitorum communis longus*
 1 Independent insertions
33. Presence of the *m. extensor brevis medius hallucis*
 0 Absent
 1 Present
34. Presence of the *m. extensor brevis medius Digiti II*
 0 Absent
 1 Present
35. Number of insertion slips of the *m. extensor brevis superficialis Digiti III*
 0 One
 1 Two
36. Relationship between the insertions of the *mm. extensor brevis superficialis Digiti III* and the *m. extensor digitorum communis longus*.
 0 Common insertion between *mm. extensor brevis superficialis Digiti III* and any slip of *m. extensor digitorum communis longus*
 1 Independent insertions
37. Origin of the *m. extensor brevis superficialis Digiti IV*
 0 By two independent slips
 1 By two proximally fused slips
 2 By one slip
38. Origin of the *m. abductor prehallucis*
 0 From the internal surface of the *aponeurosis plantaris*
 1 From the distal internal surface of the tarsus
39. Presence of the *m. abductor brevis plantaris hallucis*
 0 Absent
 1 Present
40. Type of origin of the *m. lumbricalis brevis Digiti III*
 0 Tendinous
 1 Fleshy
41. Relationship between the external slip of *m. lumbricalis brevis Digiti III* and the internal slip of *m. lumbricalis brevis Digiti V*
 0 Fused only in the origin
 1 Fused beyond its origin
- Burton characters
- Manus
42. Presence of the internal slip of *m. lumbricalis brevis Digiti III*
 0 Absent
 1 Present
43. Number of slips of the *m. lumbricalis brevis Digiti IV*
 0 Two
 1 Only one, the internal slip
- Pes
44. Origin of the *tendo superficialis hallucis*
 0 From the *aponeurosis plantaris*
 1 From the *aponeurosis plantaris* including some fibers of the *m. lumbricalis brevis hallucis*
 2 From a muscle that originates from the distal tarsal 2-3
45. Origin of the *tendo superficialis Digiti II*
 0 From the *aponeurosis plantaris*
 1 It serves as the insertion tendon of the *m. transversus plantae distalis*

Phylogenetic analysis within the *Pristimantis unistrigatus*

External characters

- 46. Skin on venter
 - 0 Areolate
 - 1 Smooth
- 47. Presence of dorsolateral folds
 - 0 Present
 - 1 Absent
- 48. Nature of tympanic anulus
 - 0 Complete
 - 1 Incomplete
- 49. Dorsal view of snout
 - 0 Rounded
 - 1 Subacuminate
 - 2 Acuminate
- 50. Shape of snout in profile
 - 0 Rounded
 - 1 Truncated
 - 2 Acute
- 51. Presence of cranial crests
 - 0 Present
 - 1 Absent
- 52. Width of the upper eyelid in relation with the inter-orbital distance
 - 0 Narrower than the inter-orbital distance
 - 1 As wide as the inter-orbital distance
- 53. Length of fingers I and II
 - 0 First finger shorter than second
 - 1 First and second finger equally or almost equally long
 - 2 First finger longer than second
- 54. Presence of finger pads or discs
 - 0 Present
 - 1 Absent
- 55. Presence of skin variations on the ulnar region
 - 0 Present
 - 1 Absent
- 56. Presence of skin variations on the heel
 - 0 Present
 - 1 Absent
- 57. Presence of supernumerary plantar tubercles
 - 0 Present
 - 1 Absent
- 58. Presence of webbed toes
 - 0 Present
 - 1 Absent
- 59. Presence of toe pads or discs
 - 0 Present
 - 1 Absent
- 60. Presence of lateral fringes on toes
 - 0 Present
 - 1 Absent