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DEFINIÇÃO TAXONÔMICA, VARIAÇÃO ACÚSTICA DE *ADENOMERA MARMORATA* STEINDACHNER, 1867 (ANURA: LEPTODACTYLIDAE) E DIVERSIDADE CRÍPTICA NAS ESPÉCIES DE MATA ATLÂNTICA DO GÊNERO *ADENOMERA* A PARTIR DE EVIDÊNCIAS MOLECULARES

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BELO HORIZONTE

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Dissertação apresentada ao programa de Pós–Graduação em Zoologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial para a obtenção do título de mestre em Zoologia.

Orientador: Prof. Dr. Paulo Christiano de Anchietta Garcia

Belo Horizonte

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Pedro Paulo Goulart Taucce

Definição taxonômica, variação acústica de *Adenomera marmorata* Steindachner, 1867 (Anura: Leptodactylidae) e diversidade críptica nas espécies de Mata Atlântica do gênero *Adenomera* a partir de evidências moleculares

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Belo Horizonte, 15 de março de 2013

Aos meus pais, Zezé e Pedro Paulo, e ao meu irmão, João.

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Maria José Goulart Taucce.

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INTRODUÇÃO GERAL

A família Leptodactylidae Werner, 1896 (*sensu* Frost, 2013), é composta por 94 espécies espalhadas em quatro gêneros: *Adenomera* Steindachner, 1867, *Hydrolaetare* Gallardo, 1963, *Leptodactylus* Fitzinger, 1826 e *Lithodites* Fitzinger, 1843. Dentre esses**,** o gênero *Adenomera* foi recentemente revalidado (Pyron e Wiens, 2011) para abrigar os membros do grupo de *Leptodactylus marmoratus* (Heyer, 1973) e hoje abriga 15 espécies formalmente descritas: *A. ajurauna* (Berneck, Costa e Garcia, 2008); *A. andreae* (Müller, 1923); *A. araucaria* Kwet e Angulo, 2002; *A. bokermanni* (Heyer, 1973); *A. coca* (Angulo e Reichle, 2008); *A. dyptix* (Boettger, 1885); *A. engelsi* Kwet, Steiner e Zilikens, 2009; *A. heyeri* Boistel, Massary e Ângulo, 2006; *A. hylaedactyla* (Cope, 1868); *A. lutzi,* Heyer, 1975; *A. marmorata* Steindachner, 1867; *A.* martinezi, (Bokermann, 1956); A*. nana* (Müller, 1922); *A. simonstuarti* (Angulo e Icochea, 2010) e *A. thomei,* (Almeida e Angulo, 2006). Sete dessas espécies habitam a Mata Atlântica Brasileira e o gênero encontra-se distribuído por toda a América do Sul, exceto na região da Patagônia. Alguns autores relatam que a riqueza de espécies do gênero pode estar subestimada (Angulo *et al.* 2003; Kokubum e Giaretta, 2005; Kwet, 2007; Angulo e Reichle, 2008; Kokubum, 2008).

O gênero *Adenomera* tem sido bastante discutido nos últimos anos. Heyer (1974) revalidou o gênero para abrigar o grupo de *Leptodactylus marmoratus* (*sensu* Heyer, 1973). Frost e colaboradores (2006), a partir de um estudo filogenético envolvendo inúmeros grupos de anfíbios, chegaram à conclusão de que o gênero *Adenomera* é sinônimo júnior do gênero *Leptodactylus*. Entretanto, esses pesquisadores haviam utilizado apenas uma espécie de *Adenomera* no trabalho (*A. hylaedacyla*) e alguns autores consideraram essa mudança taxonômica uma sinonímia precipitada (Almeida e Angulo, 2006), o que fez com que outros autores adotassem uma posição mais conservadora utilizando *Adenomera* como um gênero válido (Kwet, 2007; Ponssa, 2008). Pyron e Wiens (2011), em estudo filogenético molecular envolvendo nove genes nucleares e três genes mitocondriais, além de mais de 2800 espécies de anfíbios (sendo três do gênero *Adenomera*), recuperaram o gênero *Adenomera* em um arranjo monofilético e revalidaram-no.

A respeito da taxonomia, o primeiro estudo de revisão do gênero (Heyer, 1973) considerou cinco espécies, sendo duas delas habitantes da Mata Atlântica, *A. bokermanni* e *A. marmorata*. Porém, as duas espécies eram extremamente bem distribuídas, e alguns anos mais tarde já se pensava que poderiam ser complexos de espécies (Heyer, 1977; 1984). *Adenomera marmorata* ainda possui um agravante que deixa a taxonomia dessa espécie ainda mais complicada: sua localidade tipo é incerta, já que Stendachner (1867) definiu-a apenas como Brasil. Apesar disso, há evidências de que tal localidade seja no Estado do Rio de Janeiro (Gans, 1955) e alguns autores concordam que ela provavelmente se encontre nas proximidades da cidade do Rio de Janeiro (Bokermann, 1966; Almeida e Angulo, 2006).

Segundo Angulo e Icochea (2010), o gênero *Adenomera* possui membros que são ótimos candidatos a abrigar complexos de espécies morfologicamente crípticas (*sensu* Bickford *et al.* 2007), ou seja, duas ou mais espécies classificadas como uma única espécie. Segundo os mesmos autores, isso se dá devido à considerável variação morfológica intra e interespecífica (De La Riva, 1996; Kwet e Ângulo, 2002; Ponssa e Heyer, 2007; Kwet *et al.*

2009), à similaridade geral entre várias espécies e também porque várias das espécies nominais são consideradas amplamente distribuídas. Essa constatação é corroborada pelo fato de que mais da metade das espécies de *Adenomera* existentes foram descritas nos últimos onze anos, tendo por base, principalmente, dados bioacústicos. Estudos moleculares, porém, concernentes ao gênero, ainda não foram realizados.

Estudos envolvendo bioacústica e dados moleculares são de suma importância para a descoberta de novas espécies dentre os anfíbios (veja Heyer *et al.* 1996; Channing *et al.* 2002; Ron *et al.* 2005; Toledo *et al.* 2007; Angulo e Reichle, 2008; Padial e De la Riva, 2009; Angulo e Icochea, 2010; Padial *et al.* 2010; Fouquet *et al.* 2012). O gênero *Adenomera* representa um ótimo exemplo desse caso, uma vez que mais da metade da sua riqueza atual é fruto de descrições e revalidações recentes baseadas principalmente em dados bioacústicos.

Tendo isso em vista, o presente trabalho pretende definir *Adenomera marmorata*, apresentando dados bioacústicos e morfométricos de três de suas populações e tecendo comentários sobre o status taxonômico das mesmas. Além disso, pretende-se avaliar a diversidade críptica do gênero *Adenomera* na Mata Atlântica Brasileira tendo por base dados moleculares.

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CAPÍTULO I

Who is *Adenomera marmorata* Steindachner, 1867 (Anura: Leptodactylidae)? Bioacustics variation and definition of a problematic species

WHO IS *ADENOMERA MARMORATA* STEINDACHNER, 1867 (ANURA: LEPTODACTYLIDAE)? BIOACUSTICS VARIATION AND DEFINITION OF A PROBLEMATIC SPECIES

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1 – INTRODUCTION

The genus *Adenomera* Steindachner, 1867 was recently removed from the synonymy of *Leptodactylus* Fitzinger, 1826 (Pyron and Wiens, 2011) and comprises 15 species (Frost, 2013): *A. ajurauna* (Berneck, Costa and Garcia, 2008), *A. andreae* (Müller, 1923), *A. araucaria* Kwet and Angulo, 2002, *A. bokermanni* (Heyer, 1973), *A. coca* (Angulo and Reichle, 2008), *A. diptyx* (Boettger, 1885), *A. engelsi* Kwet, Steiner and Zillikens, 2009, *A. heyeri* Boistel, Massary and Angulo, 2006, *A. hylaedactyla* (Cope, 1868), *A. lutzi* Heyer, 1975, *A. marmorata* Steindachner, 1867, *A. martinezi* (Bokermann, 1956), *A. nana* (Müller, 1922), *A. simonstuarti* (Angulo and Icochea, 2010), and *A. thomei* (Almeida and Angulo, 2006). The genus is widely distributed throughout South America, except in the Patagonian region, and its species richness may be underestimated (Angulo, *et al.*, 2003; Kokubum and Giaretta, 2005; Kwet, 2007; Angulo and Reichle, 2008).

The genus *Adenomera* has a confusing taxonomic history. Heyer (1974) revalidated the genus to allocate the species of the *Leptodactylus marmoratus* species group*.* Frost *et al.* (2006)'s "The Amphibian Tree of Life" proposed *Adenomera* a junior synonym of *Leptodactylus*. However, they used only one species of the group at the analysis, and it was considered a precipitated synonymy (Almeida and Angulo, 2006), leading some authors to adopt a more conservative point of view using *Adenomera* as a valid genus (Kwet, 2007; Ponssa, 2008). Pyron and Wiens (2011), in a phylogenetic analysis based on nine nuclear and three mitochondrial genes of more than 2800 amphibian species, including three species of the formerly called *L. marmoratus* species group, recovered the genus *Adenomera* in a monophyletic arrangement. However, the taxonomy of the group and its phylogenetic position remain controversial.

Adenomera marmorata is a problematic species since its type locality is uncertain. The holotype, collected during the "Novara Reise" expedition, was probably found at the municipality of Rio de Janeiro or its surroundings (Bokermann, 1966; Heyer, 1973; Almeida and Angulo, 2006). However, the expedition has visited several localities within the municipality of Rio de Janeiro and even other municipalities like Petrópolis, also at the state of Rio de Janeiro (Gans, 1955; Almeida e Angulo, 2006). Considering that there are at least two species of *Adenomera* in the state of Rio de Janeiro (Almeida and Angulo, 2006), and that the genus has a considerable cryptic diversity, it is necessary to define which one of the species inhabiting the state of Rio de Janeiro is actually *A. marmorata sensu stricto*.

Adolfo Lutz (1926) described one species belonging to the genus, *Leptodactylus trivittatus*, based on exemplars of “Campo Bello” and “Alto da Serra de Cubatão”, currently municipality of Itatiaia, state of Rio de Janeiro and municipality of Santo André, state of São Paulo respectively (Bokermann, 1966). The name of the specific epithet was given due to one dorsal and two dorsolateral brick red stripes present at the specimens.

Bertha Lutz (1947) suggested that the species described by A. Lutz was a junior synonym of *Leptodactylus nanus* considering the former just a color variation of the second.

Cochran (1955) redescribed *Leptodactylus marmoratus* and considered *L. nanus* and *L. trivittatus* its junior synonyms. It was based on the fact that the coloration pattern of *L. marmoratus* is highly variable and the three species were variations of the same species.

Heyer (1973) maintained *Leptodactylus nanus* and *L. marmoratus* at the synonym of *L. marmoratus* and designed lectotypes for both of them. Concerning *L. trivittatus,* the chosen specimen was a juvenile, because it was the only one among the sintypes that showed the striped pattern that named the species. However, the author was confused when placed the type locality as “Campo Bello, Alto da Serra de Cubatão, Brasil”, since the two localities are different places, in different mountainous complexes (Serra da Mantiqueira and Serra do Mar respectively). Cochran (1955) examined the specimen that would later be defined as the lectotype of *L. trivittatus* and specified the collection site of the specimen: “Montserrat, Campo Bello, Rio de Janeiro”, which is currently the headquarters of the Itatiaia National Park, municipality of Itatiaia. Therefore, the name *L. trivittatus,* if valid, belongs to the Itatiaia population.

More recently, Kwet (2007) revalidated *Leptodactylus nanus* and disagreed with B. Lutz (1947), considering more likely that *L. trivittatus* was a junior synonym of *L. marmoratus*, because of the great geographic distance separating *L. nanus* and *L. trivittatus.*

The anuran advertisement call is an important pre-mating isolation mechanism (Duellman and Trueb, 1994; Kelley *et al.* 2001). Analyzing its temporal and spectral parameters is of paramount importance in elucidating the taxonomy of the anuran species, mainly in crypt or polymorphic groups, as on the case of *Adenomera* (Heyer, 1984; Kwet and Angulo, 2002; Angulo *et al.* 2003; Kwet, 2007).

Herein we define and present a diagnosis for *Adenomera marmorata*, describe the advertisement call of three populations of the species, and give comments about the taxonomic status of them.

2 – MATERIALS AND METHODS

Advertisement calls from three populations and 41 specimens of *Adenomera marmorata* in Rio de Janeiro and São Paulo states were recorded during field studies from 2004–2012 (localities shown at Appendix I) and some of them were used in another study (see Berneck *et al.* 2008). Voucher specimens for these calls are deposited at Coleção Herpetológica da Universidade Federal de Minas Gerais (UFMG). The specimens of some recordings could not be collected, but for all populations at least one conspecific specimen was collected.

Specimens examined for measurements and morphological comparisons are housed in the following collections: Coleção Herpetológica da Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil; Museu Nacional do

Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil; and Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil. The following measurements were made to the nearest 0.01 mm using a digital caliper: snout vent length (SVL), head length (HL), head width (HW), tibia length (TL), foot length (FL) (Duellman, 2001), forearm length (FAL) (Duellman *et al.* 1997), hand length (HAL), thigh length (THL), and tarsal length (TAL) (Heyer *et al.* 1990). Eye diameter (ED), tympanum diameter (TD), eye to nostril distance (END), internarial distance (IND) (Duellman, 2001), and distance between the anterior margins of eyes (AMD) (Garcia *et al.* 2003) were taken with an ocular micrometer fitted to a stereomicroscope. Measurements of the holotype of *Adenomera marmorata* were taken by Kwet and Angulo (2002).

Call recordings were made with a Marantz PMB-222 tape record with an Audiotechnica microphone AT 835, or with a Marantz PMD 660 or a Marantz PMD 661 digital recorders, both with a Sennheiser K6 super–cardioid shotgun condenser microphone system with an ME66 module. Analogical recordings were digitized at 44.1 kHz and digital recordings were sampled with a frequency rate of 16 bits. The recordings were analyzed with the program Raven 1.4 pro for Windows (Cornell Lab of Ornithology Research Program Bioacoustics Workstation). The spectrograms were produced with FFT of 256 points, overlap 75% and window Hann. Resolution, contrast and brightness settings were the program default. Measurements of the following acoustic parameters were taken: call duration, dominant frequency range, interval between calls and call rate. They all follow Cocroft and Ryan (1995). Peak frequency is a parameter obtained directly from the software. Temporal parameters were measured in seconds (s) and spectral parameters in Hertz (Hz).

Advertisement calls were divided in three groups, taking into account the geographic location they were recorded and are shown at Appendix I.

A bloxpot graphic was used to show the variance within the call duration and a Spearman correlation test was used to analyze the relationship between that parameter and air temperature. The tests were held in the software R (R Development Core Team, 2012).

3 – RESULTS

3.1 – Advertisement calls

Males were found calling in the evening and at night between the leaf–litter in forest edges. The advertisement calls of all specimens present in this study consists in one unpulsed note emitted several times per minute, as most species of *Adenomera* (Boistel *et al.* 2006; Kwet, 2006; Kwet, 2007; Berneck *et al.* 2008, Kwet *et al.* 2009). It also presented harmonic structure, and the fundamental frequency was the dominant frequency. The three groups analyzed in this study differ primarily in temporal parameters (Appendix II, Fig. 1). The call duration, although some overlap, differed significantly (Fig. 2) between the populations and showed a significant ($p < 0.05$) and positive ($r = 0.39$) correlation with air temperature. A description of temporal and spectral parameters of the advertisement calls of each group is presented below.

3.1.1 – Parque Nacional do Itatiaia and its sorroundings (Appendix II and Fig. 1A):

Call duration (n = 170 calls in 17 individuals) ranged from 0.087 to 0.139 s (mean = 0.106 ± 0.014 s) and interval between notes from 0.792 to 1.976 (mean = 1.289 ± 0.359 s), with 32 to 74 (mean = 50 ± 12) calls per minute.

Peak frequency varied from 3789.8 to 4995.7 Hz and the dominant frequency from 3435.2 to 5516.6 Hz.

3.1.2 – Paranapiacaba and Bertioga (Appendix II and Fig. 1B):

Call duration (n = 139 in 14 individuals) ranged from 0.026 to 0.057 s (mean = 0.037 ± 0.011 s) and interval between notes from 0.756 to 1.811 s (mean = 1.192 ± 0.282 s), with 37 to 84 (mean = 57 ± 13) calls per minute. Peak frequency varied from 4651.2 to 5081.8 Hz and the dominant frequency from 4139.9 to 5620.1 Hz.

3.1.3 – Municipality of Rio de Janeiro and its surroundings (Appendix II and Fig. 1C):

Call duration (n = 94 in 10 individuals) ranged from 0.036 to 0.091 s (mean = 0.066 ± 0.016 s) and interval between notes from 1.148 to 4.112 s (mean = 2.217 ± 0.873 s), with 16 to 55 (mean = 33 ± 12) calls per minute. Peak frequency varied from 4306.6 to 4823.4 Hz and the dominant frequency from 3786.3 to 5470.8 Hz.

Comparisons between the advertisement calls of the three populations of *Adenomera marmorata* and other *Adenomera* species are presented in Table 2.

3.2 – Morphological characters

There are no markedly morphometric differences between the three populations analyzed in this study (Table 2). Berneck *et al.* 2008 argue that none of the specimens from Rio de Janeiro, RJ analyzed in their study have the stripe pattern mentioned by Lutz (1926), but they consider that perhaps this is due to a small sampling. We have found specimens with the pattern in the municipalities of Rio de Janeiro, Petrópolis and Guapimirim, as well as in other municipalities present in our study (Fig. 3).

3.3 – Diagnosis

Adenomera marmorata is a medium sized *Adenomera* (SVL in males 17.36–21.60 mm, mean 20.03; see Table 1) and differs from other species from the Brazilian Atlantic rain forest as follows: (1) from the partly sympatric occurring *A. ajurauna* by its bigger size (males maximum SVL 19.95 mm in *A. ajurauna*, Berneck *et al.* 2008), light colored throat (dark brown in *A. ajurauna*), and advertisement call; (2) from *A. araucaria* by its bigger size (males maximum SVL 18.8 mm in *A. araucaria*, Kwet and Angulo, 2002) and unpulsed advertisement call (pulsed in *A. araucaria*); (3) from *A. bokermanni* by its smaller size (males maximum SVL 25.1 mm in *A. bokermanni*, Heyer, 1973) and flattened toe tips; (4) from *A. engelsi* by the less acuminate snout profile and advertisement call (see Kwet *et al*. 2009); (5) from *A. nana* by the slightly bigger size (males maximum SVL 19.4 mm in *A. nana*, Kwet, 2007) and advertisement call; (6) and from *A. thomei* by the flattened toe tips, terrestrial reproductive mode, and unpulsed advertisement call (pulsed in *A. thomei*).

4 – DISCUSSION

The calls of the three populations of *Adenomera marmorata* differed mainly on temporal parameters (Appendix II, Fig. 1, Fig. 2). Temporal properties are highly temperature-dependant, and spectral properties seem to be much more stable concerning temperature (Gerhardt and Mudry, 1980). Call duration may be highly variable within the same species and females show little selectivity towards it (Doherty and Gerhardt, 1984). Another possibility that may explain the variation of the call duration in *A. marmorata* is the Reproductive

Character Displacement hypothesis (Brown and Wilson, 1956; Grant, 1972), which is the change of a character state of a species, under natural selection, by the presence in the same environment of one or more species reproductively similar to the first one. There are some studies supporting the hypothesis with frogs (Ralin, 1977; Gerhardt, 1994; Hoskin *et al.,* 2005), flyes (Wasserman and Koepfer, 1977), and even plants (Muchhala and Potts, 2007). The only known populations of *A. marmorata* living with a syntopic species are Paranapiacaba and Bertioga populations, which can be found calling at very close sites with *A. ajurauna* (Taucce and Garcia pers. com.). They have the shortest call duration (0.026–0.057 s) within *A. marmorata,* while the advertisement call of *A. ajurauna* is considerably longer (0.130–0.190 s). We conclude then that probably the short call of *A. marmotata* in these places may be due reproductive character displacement. Assuming that all and our morphological data, we understand that the three populations are conspecifc.

Kwet (2007) revalidated *Leptodactylus nanus* for the southern populations of *A. marmorata.* The calls of the two species are quite similar, but *A. nana* have a fundamental frequency range between 2300–2800 Hz, which is different from its dominant frequency range (Kwet, 2007; Table 2). The two species have similar dominant frequency ranges, but in *A. marmorata* it is the same as the fundamental frequency. *A. nana* is also slightly smaller (see diagnosis) and the two species are separated by a considerable geographic distance (Fig. 4). Thus we agree with Kwet (2007) and consider *A. nana* as a valid taxon.

Adenomera marmorata is a problematic species (see introduction). There are at least three species of *Adenomera* in the state of Rio de Janeiro. One of them is the one present in this study. There is another species in the municipality of Itatiaia, but it has a pulsed advertisement call and is more related to *A. thomei* (Cassini *et al.* in prep.). It has not the striped pattern mentioned by Lutz (1926), and since the lectotype of *Leptodactylus trivittatus* has the pattern, they are not the same species. The other one is mentioned by Almeida and Angulo (2006) for Teresópolis, RJ, which is very near Petrópolis, RJ (one of the localities visited by the “Novara Reise” expedition) and it has the advertisement call markedly different from the species we analyzed in the present study (see Berneck *et al.* 2008 for acoustic parameters). Almeida and Angulo (2006) say that it is crucial to know the specific type locality of *A. marmorata* to elucidate the taxonomic status of the species. But this may be an impossible task. Among the places visited by the expedition, the species analyzed in the present study is extremely abundant in Laranjeiras and Tijuca, in the municipality of Rio de Janeiro, and in the forest edges of the municipality of Petrópolis (Taucce and Garcia, pers. obs.). Therefore we consider that it is unlikely that the holotype of *A. marmorata* is not conspecific with the species present in this study. Based on that and the fact that the holotype collected during the “Novara Reise” expedition morphologically corresponds with the species present in this study, for now on we propose that *Adenomera marmorata* is the most abundant species in the municipality of Rio de Janeiro, and agree with Bokermann (1966) and Almeida and Angulo (2006) regarding the type locality of the species. We also agree with Cochran (1955), Heyer (1973) and Kwet (2007) about *Leptodactylus trivittatus* Lutz, 1926 being a junior synonym of *Adenomera marmorata* Steindachner, 1867. *A. marmorata* is distributed along the Brazilian Atlantic forest in the states of São Paulo and Rio de Janeiro, from the sea level to 1100 meters of altitude (fig. 4). Besides our data, we also included in the distribution map localities that have described advertisement calls (Heyer *et al.*, 1990; Haddad *et al.* 2005) that corresponds to the one described in this study (Appendix I).

The original description of *Adenomera marmorata* (Steindachner, 1867) is the first one for the genera and fits most of described *Adenomera* species. The most important characteristic with taxonomic value present in the description is the shallow toe tips, but it does not mention if it is flattened or not. We understand that the two available redescriptions for *A. marmorata* (Cochran, 1955 and Heyer, 1973), despite being the case of a complex of species, they are accurate and a new redescription is not necessary. A diagnosis (Item 3.3) was presented to help elucidating the taxonomic status of *A. marmorata.*

5 – CONCLUSION

Adenomera marmorata corresponds to the most abundant species of *Adenomera* present in the municipality of Rio de Janeiro, state of Rio de Janeiro, which is its probable type locality, and it is distributed throughout the Brazilian Atlantic rainforest of the states of São Paulo and Rio de Janeiro. The advertisement call is highly variable as regards the call duration. There are still not described species in southeastern Brazil (Almeida and Angulo, 2006; this study) and studies like this are important to help elucidating the taxonomy of the genus *Adenomera*. Molecular studies are necessary to explain the evolution and biogeography of *A. marmorata*.

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7 – ABSTRACT

The genus *Adenomera* comprises 15 species distributed through South America, except in the Patagonian Region. Within them, *A. marmorata* is a problematic species since its type locality is uncertain. The advertisement call of three *A. marmorata* populations is described based on specimens collected at six localities of the states of Rio de Janeiro and São Paulo, including the putative type locality of the species. As well as most *Adenomera* species, the advertisement call of *A. marmorata* consists in one short and unpulsed note emitted several times per minute with harmonical structure. The three groups analyzed in this study differed mainly in the call duration parameter, which is positively correlated with temperature. Bioacoustics, literature and field data were used to define *A. marmorata* and to make conclusions about its putative type locality. We concluded that *A. marmorata* corresponds to the most abundant species of *Adenomera* present in the municipality of Rio de Janeiro, which is the probable type locality of the species, and is distributed throughout the states of Rio de Janeiro and São Paulo. We agreed with previous studies,

concluding that *Leptodatylus trivitattus* is a junior synonym of *A. marmorata* and *Leptodactylus nanus* is a valid species.

8 – RESUMO

O gênero *Adenomera* compreende 15 espécies distribuídas por toda a América do Sul, exceto na região Patagônica. Dentre elas, *A. marmorata* é uma espécie problemática tendo em vista que sua localidade tipo é incerta. O canto de anúncio de três populações de *A. marmorata* é descrito com base em espécimes coletados em seis localidades nos estados do Rio de Janeiro e São Paulo, incluindo a provável localidade tipo da espécie. Assim como a maioria das espécies de *Adenomera*, o canto de anúncio de *A. marmorata* consiste em uma nota curta e não–pulsionada emitida várias vezes por minuto com estrutura harmônica. Os três grupos analisados neste estudo diferem principalmente na duração do canto, que se correlaciona positivamente com a temperatura. Dados de bioacústica, de literatura e de campo foram utilizados para definir *A. marmorata* e tirar conclusões sobre sua provável localidade tipo. Concluiu-se que *A. marmorata* corresponde a espécie mais abundantes de *Adenomera* presente no município do Rio de Janeiro, que é a provável localidade tipo da espécie, e é distribuída pelos estados do Rio de Janeiro e São Paulo. Nós concordamos com estudos anteriores, concluindo que *Leptodatylus trivitattus* é sinônimo júnior de *A. marmorata* e *Leptodactylus nanus* é uma espécie válida.

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APPENDIX I: RECORDINGS

Paranapiacaba and Bertioga (one of the type localities of *L. trivittatus* designated by Lutz, 1926):

(1) Paranapiacaba, Santo André, SP, recording PPGT006, call voucher UFMG9469, 20 November 2011, 19.50h Brazilian Daylight saving time, 18ºC air temperature.

(2) Paranapiacaba, Santo André, SP, recording PPGT009, call voucher UFMG9471, 20 November 2011, 21.50h Brazilian Daylight saving time, 18ºC air temperature.

(3) Paranapiacaba, Santo André, SP, recording PPGT002, unvouchered, 20 November 2011, 18.20h Brazilian Daylight saving time, 19ºC air temperature.

(4) Paranapiacaba, Santo André, SP, recording PPGT003, unvouchered, 20 November 2011, 19.10h Brazilian Daylight saving time, 18ºC air temperature.

(5) Paranapiacaba, Santo André, SP, recording PPGT005, unvouchered, 20 November 2011, 19.30h Brazilian Daylight saving time, 18ºC air temperature.

(6) Paranapiacaba, Santo André, SP, recording PPGT012, unvouchered, 21 November 2011, 19.15h Brazilian Daylight saving time, 18ºC air temperature.

(7) Paranapiacaba, Santo André, SP, recording PPGT013, unvouchered, 21 November 2011, 19.45h Brazilian Daylight saving time, 18ºC air temperature.

(8) Paranapiacaba, Santo André, SP, recording PPGT015, unvouchered, 21 November 2011, 21.30h Brazilian Daylight saving time, 18ºC air temperature.

(9) Parque das Neblinas, Bertioga, SP, recording Paulo C. A. Garcia, unvouchered, 07 November 2006.

(10) Parque das Neblinas, Bertioga, SP, recording Paulo C. A. Garcia, unvouchered, 21 December 2005.

(11) Parque das Neblinas, Bertioga, SP, recording Paulo C. A. Garcia, unvouchered, 25 September 2005.

(12) Parque das Neblinas, Bertioga, SP, recording Paulo C. A. Garcia, unvouchered, December, 2004.

(13) Parque das Neblinas, Bertioga, SP, recording Paulo C. A. Garcia, unvouchered, November 2004.

(14) Parque das Neblinas, Bertioga, SP, recording Paulo C. A. Garcia, unvouchered, November, 2004.

Three of the advertisement calls numbers nine to fourteen were analyzed at Berneck *et al.* 2008, but we cannot say which of them. There are also two voucher specimens for the calls, MZUSP136703 and MZUSP136760, according to the study. We are also not able to link the vouchers to a precise recording.

Municipality of Rio de Janeiro and its surroundings (presumed type locality of *Adenomera marmorata*):

(1) Parque Nacional da Tijuca, Rio de Janeiro, RJ, Carla S. Cassini recording, unvouchered

(2) Parque Nacional da Tijuca, Rio de Janeiro, RJ, Carla S. Cassini recording, unvouchered

(3) Parque Nacional da Tijuca, Rio de Janeiro, RJ, Carla S. Cassini recording, call voucher CFBH

(4) Parque Nacional da Tijuca, Rio de Janeiro, RJ, Carla S. Cassini recording, unvouchered

(5) Petrópolis, RJ, recording PPGT079, unvouchered, 27 January 2012, 21.50h Brazilian daylight saving time, 20ºC air temperature.

(6) Petrópolis, RJ, recording PPGT082, call voucher UFMG10296, 27 January 2012, 22.20h Brazilian daylight saving time, 20ºC air temperature.

(7) Guapimirim, RJ, recording PPGT084, unvouchered, 29 January 2012, 20.50h Brazilian daylight saving time, 17.5ºC air temperature.

(8) Guapimirim, RJ, recording PPGT087, unvouchered, 29 January 2012, 22.20h Brazilian daylight saving time, 18ºC air temperature.

(9) Guapimirim, RJ, recording PPGT086, call voucher UFMG10303, 29 January 2012, 21.15h Brazilian daylight saving time, 17.5ºC air temperature.

(10) Guapimirim, RJ, recording PPGT085, call voucher UFMG10304, 29 January 2012, 21.00h Brazilian daylight saving time, 17.5ºC air temperature.

Parque Nacional do Itatiaia and its surroundings (type locality of *Leptodactylus trivittatus* designated by Heyer, 1973):

(1) Itatiaia, RJ, Carla S. Cassini recording, unvouchered, 02 November 2011.

(2) Itatiaia, RJ, Carla S. Cassini recording, call voucher UFMG9353, 03 November 2011.

(3) Itatiaia, RJ, Carla S. Cassini recording, call voucher UFMG9354, 04 November 2011.

(4) Itatiaia, RJ, Carla S. Cassini recording, unvouchered, 05 November 2011.

(5) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT102, call voucher PPGT140, 14 December 2012, 20.30h Brazilian daylight saving time ,19ºC air temperature.

(6) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT103, call voucher PPGT141, 14 December 2012, 21.00h Brazilian daylight saving time, 19ºC air temperature.

(7) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT105, unvouchered, 15 December 2012, 18.10h Brazilian daylight saving time, 20ºC air temperature.

(8) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT106, unvouchered, 15 December 2012, 18.30h Brazilian daylight saving time, 21ºC air temperature.

(9) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT107, unvouchered, 15 December 2012, 18.45h Brazilian daylight saving time, 21ºC air temperature.

(10) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT110, unvouchered, 15 December 2012, 20.30h Brazilian daylight saving time, 21ºC air temperature.

(11) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT111, unvouchered, 15 December 2012, 21.50h Brazilian daylight saving time, 20ºC air temperature.

(12) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT112, unvouchered, 15 December 2012, 22.10h Brazilian daylight saving time, 20ºC air temperature.

(13) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT113, call voucher PPGT145, 16 December 2012, 19.00h Brazilian daylight saving time, 18,5ºC air temperature.

(14) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT114, unvouchered, 16 December 2012, 19.20h Brazilian daylight saving time, 18,5ºC air temperature.

(15) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT115, call voucher PPGT146, 16 December 2012, 19.40h Brazilian daylight saving time, 17ºC air temperature.

(16) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT116, unvouchered, 16 December 2012, 20.00h Brazilian daylight saving time, 17ºC air temperature.

(17) Parque Nacional do Itatiaia, Itatiaia, RJ, recording PPGT117, call voucher PPGT148, 16 December 2012, 22.00h Brazilian daylight saving time, 21ºC air temperature.

APPENDIX II: BIOACOUSTIC PARAMETERS OF THE SPECIMENS OF *ADENOMERA MARMORATA*

Specimen	Locality	Air Temperature (°C)	Number of analyzed calls	Call duration (s)	Peak Frequency (Hz)	Dominant frequency range (Hz)	Interval between calls (s)	Call rate
Not collected	Itatiaia, RJ	—	10	0.084–0.108 (0.091±0.003)	4500.0	3896.4–5330.7	0.744–1.336 (0.979±0.197)	57
UFMG9353	Itatiaia, RJ	—	10	0.102–0.120 (0.114±0.005)	4500.0	3872.5–5354.6	1.419–2.233 (1.654±0.275)	40
UFMG9354	Itatiaia, RJ	—	10	0.124–0.145 (0.129±0.007)	4312.5 and 4500.0	3346.6–5426.3	0.551–1.272 (0.893±0.266)	58
Not collected	Itatiaia, RJ	—	10	0.097–0.101 (0.100±0.001)	4478.9 and 4651.2	3997.1–5161.1	0.834–1.106 (0.941±0.088)	59
UFMG13283	Itatiaia, RJ	19	10	0.099–0.116 (0.105±0.005)	4478.9 and 4651.2	3936.0–5193.1	1.232–1.934 (1.593±0.256)	39
UFMG13284	Itatiaia, RJ	19	10	0.099–0.108 (0.104±0.003)	4306.6	3812.4–4842.8	0.794–1.244 (0.922±0.153)	65
Not collected	Itatiaia, RJ	20	10	0.104–0.109 (0.107±0.002)	4823.4 and 4995.7	4183.3–5543.4	0.725–0.944 (0.792±0.065)	74
Not collected	Itatiaia, RJ	21	10	0.094–0.101 (0.096±0.003)	4478.9 and 4651.2	4100.9–5193.1	0.745–1.330 (1.014±0.185)	60
Not collected	Itatiaia, RJ	21	10	0.081–0.092 (0.087±0.003)	4306.6 and 4478.9	3730.0–5069.4	0.799–1.190 (0.969±0.157)	63
Not collected	Itatiaia, RJ	21	10	0.093–0.104 (0.098±0.004)	4651.2 and 4478.9	3915.4–5193.1	1.116–2.415 (1.539±0.469)	41
Not collected	Itatiaia, RJ	20	10	0.093–0.108 (0.102±0.004)	4134.4; 4306.6 and 4478.9	3462.1–4822.1	1.287–2.709 (1.685±0.433)	37
Not collected	Itatiaia, RJ	20	10	0.099–0.104 (0.102±0.002)	4134.4	3585.7–4677.9	0.977–1.139 (1.084±0.055)	56
UFMG13288	Itatiaia, RJ	18.5	10	0.126–0.145 (0.139±0.006)	3789.8 and 3962.1	3400.2–4348.2	1.157–1.814 (1.487±0.212)	41

Specimen	Locality	Air Temperature (°C)	Number of analyzed calls	Call duration (s)	Peak Frequency (Hz)	Dominant frequency range (Hz)	Interval between calls (s)	Calls/min
Not collected	Itatiaia, RJ	18.5	10	0.124–0.133 (0.128±0.003)	3962.1	3565.1–4554.3	1.216–1.583 (1.421±0.118)	43
UFMG13289	Itatiaia, RJ	17	10	0.084–0.094 (0.090±0.003)	4306.6	3812.4–4842.8	1.348–2.203 (1.621±0.295)	39
Not collected	Itatiaia, RJ	17	10	0.099–0.108 (0.104±0.003)	4134.4 and 4306.6	3647.5–4863.4	1.595–2.687 (1.976±0.392)	32
UFMG13291	Itatiaia, RJ	21	10	0.099–0.111 (0.107±0.004)	4478.9 and 4651.2	3771.2–5337.3	1.184–1.541 (1.349±0.108)	46
UFMG9469	Santo André, SP	18	10	0.036–0.040 (0.039±0.001)	4651.2 and 4823.4	4106.9–5446.6	1.331–2.125 (1.543±0.280)	41
UFMG9471	Santo André, SP	18	10	0.035–0.039 (0.037±0.001)	4651.2	4085.0–5336.8	1.132–2.010 (1.494±0.253)	42
Not collected	Santo André, SP	19	10	0.031–0.037 (0.034±0.002)	4823.4	4287.2–5385.3	0.870–1.374 (1.157±0.172)	54
Not collected	Santo André, SP	18	10	0.025–0.027 (0.026±0.001)	4995.7	4612.1–5556.4	1.221–1.623 (1.352±0.117)	47
Not collected	Santo André, SP	18	9	0.022–0.030 (0.027±0.003)	4651.2	4260.7–5512.5	1.395–2.370 (1.811±0.338)	37
Not collected	Santo André, SP	18	10	0.031–0.034 (0.033±0.001)	4823.4	4392.4–5556.4	0.666–0.950 (0.756±0.085)	85
Not collected	Santo André, SP	18	10	0.043–0.047 (0.045±0.001)	4823.4 and 4995.7	4260.7–5732.1	0.802–1.702 (1.093±0.284)	59
Not collected	Santo André, SP	18	10	0.029–0.033 (0.031±0.001)	4823.4	4348.5–5556.4	0.936–1.304 (1.133±0.105)	57
See Appendix I	Bertioga, SP	—	10	0.029–0.032 (0.030±0.001)	4651.2 and 4737.3	4392.4–5095.2	1.102–1.386 (1.223±0.106)	53
See Appendix I	Bertioga, SP	—	10	0.054–0.060 (0.057±0.002)	4737.3 and 4823.4	4414.4–5249.0	0.916–1.553 (1.167±0.218)	55

Specimen	Locality	Air Temperature (ºC)	Number of analyzed calls	Call duration (s)	Peak Frequency (Hz)	Dominant frequency range (Hz)	Interval between calls (s)	Calls/min
See Appendix I	Bertioga, SP	—	10	0.023–0.029 (0.026±0.002)	4737.3 and 4823.4	4403.4–5314.8	0.703–1.058 (0.848±0.115)	76
See Appendix I	Bertioga, SP	—	10	0.029–0.039 (0.031±0.003)	5081.8	4820.7–5512.5	0.817–1.171 (0.921±0.116)	70
See Appendix I	Bertioga, SP	—	10	0.048–0.054 (0.051±0.002)	4651.2 and 4737.3	4370.5–5029.3	0.733–1.241 (1.093±0.156)	58
See Appendix I	Bertioga, SP	—	10	0.047–0.059 (0.054±0.004)	4651.2	4348.5–5161.1	0.950–1.332 (1.097±0.117)	58
Not collected	Rio de Janeiro, RJ	—	10	0.066–0.081 (0.075±0.005)	4478.9 and 4823.4	3887.3–5534.5	1.041–1.305 (1.148±0.102)	55
Not collected	Rio de Janeiro, RJ	—	5	0.052–0.060 (0.057±0.003)	4478.9 and 4651.2	3799.5–5095.2	2.435–3.104 (2.693±0.300)	22
CFBH27850	Rio de Janeiro, RJ	—	10	0.032–0.040 (0.036±0.003)	4306.6	3733.6–5161.1	0.833–3.320 (1.490±0.727)	44
Not collected	Rio de Janeiro, RJ	—	10	0.045–0.050 (0.047±0.002)	4306.6 and 4478.9	3777.5–5314.8	1.331–10.826 (4.112±2.842)	16
Not collected	Petrópolis, RJ	20	10	0.070–0.074 (0.072±0.001)	4306.6 and 4478.9	3870.5–5132.3	1.491–1.943 (1.774±0.143)	36
UFMG10296	Petrópolis, RJ	20	10	0.044–0.057 (0.051±0.005)	4478.9	3909.3–5512.5	1.280–1.444 (1.370±0.063)	36
Not collected	Guapimirim, RJ	17.5	10	0.069–0.080 (0.074±0.004)	4306.6 and 4478.9	3777.5–5512.5	1.963–2.951 (2.253±0.302)	29
Not collected	Guapimirim, RJ	17.5	9	0.054–0.065 (0.060±0.003)	4478.9 and 4651.2	3953.2–5534.5	1.101–9.946 (2.446±3.036)	27
UFMG10303	Guapimirim, RJ	17.5	10	0.070–0.081 (0.073±0.002)	4306.6 and 4478.9	3821.4–5336.8	1.077–3.526 (2.060±0.886)	31
UFMG10304	Guapimirim, RJ	18	10	0.086–0.095 (0.089±0.002)	4306.6	3755.5–5336.8	1.658–3.993 (2.828±0.792)	23
Heyer *et al.*1990	Boracéia, Salesópolis, SP	18	16	0.040–0.070	–	4500.0–5400.0	–	48–84

Specimen	**Locality**	**Air Temperature (°C)**	**Number of analyzed calls**	**Call duration (s)**	**Peak Frequency (Hz)**	**Dominant frequency range (Hz)**	**Interval between calls (s)**	**Calls/min**
Haddad *et al.* 2005	Ubatuba, SP	20.5	9	0.090–0.100	–	4300.0–4600.0	–	70

APPENDIX III: SPECIMENS EXAMINED

State of Rio de Janeiro: Itatiaia, UFMG 9353, 9354, 13283–13286, 13288–13291; Petrópolis: MNRJ 73979, UFMG 10296, 10297; Guapimirim, UFMG 10298–10304; Rio de Janeiro, MNRJ 27653, 43072, 43073. State of São Paulo: Bertioga, UFMG 2708; Boracéia: MZUSP 2733, 4061, 4062, 24144, 14145, 24147, 24295–24297, 24310, 24313, 68984; Paranapiacaba, MZUSP 1865, UFMG 9469–9471.

TABLE 1. Condensed data of acoustic parameters of Brazilian Atlantic rainforest *Adenomera* species with described advertisement calls. ES, state of Espírito Santo, RJ, state of Rio de Janeiro, RS, state of Rio Grande do Sul, SC, state of Santa Catarina, SP, state of São Paulo. Brazil.

TABLE 2. Morphometric parameters for the holotype of *Adenomera marmorata* and three populations of the species. Data are presented like this: Min–max; (mean±standard deviation).

FIG. 1. Advertisement call of *Adenomera marmorata*. (A) Waveform (above) and spectrogram (below) of a specimen from Paranapiacaba, Santo André, SP. Recording PPGT009, call voucher UFMG9471, 20 November 2011, 21.50h Brazilian Daylight saving time, 18ºC air temperature. (B) Waveform (above) and spectrogram (below) of a specimen from Petrópolis, RJ. recording PPGT082, call voucher UFMG10296, 27 January 2012, 22.20h Brazilian daylight saving time, 20ºC air temperature. (C) Waveform (above) and spectrogram (below) of a specimen from Itatiaia, RJ. Recording PPGT117, call voucher PPGT148, 16 December 2012, 22.00h Brazilian daylight saving time, 21ºC air temperature.

Fig. 2. Boxplot showing differences in call duration of the three populations of *Adenomera marmorata* analyzed in this study. 1 = Paranapiacaba and Bertioga, state of São Paulo; 2 = Rio de Janeiro and its surroundings, state of Rio de Janeiro; and 3 = Parque Nacional do Itatiaia and its sorroundings, state of Rio de Janeiro.

Fig. 3. *Adenomera marmorata* specimens showing the striped pattern mentioned by Lutz (1926). All the specimens are adult males. (A) UFMG2708, Bertioga, SP (B) UFMG9471, Paranapiacaba, Santo André, SP. (C)

MNRJ43073, Rio de Janeiro, RJ. (D) UFMG10304, Guapimirim, RJ. (E) UFMG10296, Petrópolis, RJ. (F) UFMG13291, Itatiaia, RJ. Scale bar = 10 mm.

Fig. 4. Geographic distribution of *Adenomera marmorata* (circles) and *A. nana* (triangles) based on populations with known advertisement calls. Dark geometric figures are type localities. 1 – Rio de Janeiro, 2 – Guapimirim, 3 – Petrópolis, 4 – Itatiaia, 5 – Ubatuba, 6 – Boracéia, Salesópolis, 7 – Paranapiacaba, Santo André, 8 – Bertioga, 9 – Pirabeiraba, 10 – Guaramirim, 11 – Jaraguá do Sul, 12 – Corupá, 13 – Morro do Baú, 14 – Blumenau, 15 – Rodeio, 16 – Ibirama. SP, state of São Paulo, MG, state of Minas Gerais, RJ, state of Rio de Janeiro, PR, state of Paraná, SC, state of Santa Catarina. Brazil.

TABLE 1.

	Paranapiacaba, Bertioga, and Boraceia, SP (n = 17)	Rio de Janeiro, RJ and sorroundings (n = 13)	PNI and surroundings (n = 10)	NHW14653 (Holotype of *Adenomera marmorata*)
SVL	17.36–21.60 (19.34±1.31)	18.26–21.31 (20.32±0.82)	19.07–21.58 (19.97±0.85)	21.60
HL	5.32–8.04 (6.89±0.76)	7.28–8.45 (7.93±0.38)	7.84–9.01 (8.34±0.39)	7.70
HW	5.40–6.76 (6.08±0.40)	6.35–7.83 (7.10±0.43)	6.91–7.46 (7.08±0.17)	7.40
ED	1.42–2.59 (2.00±0.31)	1.91–2.24 (2.05±0.09)	1.97–2.30 (2.10±0.10)	–
TD	0.82–1.56 (1.18±0.21)	0.82–1.21 (1.00±0.11)	1.00–1.18 (1.10±0.06)	–
END	1.21–2.27 (1.67±0.27)	1.27–1.76 (1.54±0.12)	1.27–1.55 (1.41±0.08)	–
IND	1.38–2.36 (1.90±0.27)	1.76–2.09 (1.92±0.08)	1.73–2.00 (1.85±0.11)	–
AMD	0.58–3.63 (3.05±0.68)	3.09–3.67 (3.42±0.20)	3.27–3.73 (3.55±0.17)	–
FAL	2.88–4.74 (3.80±0.53)	3.43–4.47 (3.96±0.31)	3.54–4.20 (3.86±0.27)	–
HAL	3.52–4.99 (4.27±0.39)	4.44–5.28 (4.76±0.22)	4.76–6.00 (5.12±0.36)	–
THL	6.74–9.24 (7.70±0.70)	7.75–9.60 (8.66±0.45)	8.19–9.94 (9.02±0.48)	9.20
TL	7.43–9.66 (8.81±0.58)	9.00–10.24 (9.55±0.38)	9.03–10.48 (9.69±0.42)	9.60
TAL	4.96–6.26 (5.47±0.41)	4.94–6.02 (5.35±0.34)	5.01–6.03 (5.49±0.32)	–
FL	7.34–11.22 (9.81±0.99)	9.46–11.33 (10.58±0.58)	10.91–12.68 (11.43±0.54)	–

TABLE 2.

Species	Adenomera ajurauna (Berneck *et al.* 2008)	Adenomera araucaria (Kwet, 2007)	Adenomera engelsi (Kwet *et al.* 2009)	Adenomera marmorata (this study)	Adenomera nana (Kwet, 2007)	Adenomera thomei (Almeida & Angulo, 2006)
Locality	Bertioga, SP	Several municipalities in the states of SC and RS	Several municipalities in the state of SC	States of Rio de Janeiro and São Paulo	Several municipalities in the state of SC	Linhares, ES
Air Temperature (^{0}C)	18.3–19.2	16–28.5	17–25.5	17–21	17–22	17.7–19.7
Number of analyzed calls	40	120	62	303	108	76
Pulses per call	1	6–18	1	1	1	10–21
Call duration (s)	0.130–0.190	0.064–0.220	0.096–0.163	0.026–0.139	0.055–0.122	0.120–0.210
Peak Frequency (Hz)	–	–	–	3789.8–5081.8	–	–
Dominant frequency range (Hz)	3723.0–5425.0	4200.0–5650.0	3460.0–4290.0	3435.2–5620.1	4620.0–5400.0	4565.5–5562.3
Fundamental frequency range (Hz)	Same as dominant frequency	2200.0–2980.0	Same as dominant frequency	Same as dominant frequency	2300.0–2800.0	2153.3–2811.1
Interval between calls (s)	–	–	–	0.756–4.112	–	–
Call rate (calls/minute)	23–36	22–72	10–24	16–85	22–37	14–23

FIGURE 1.

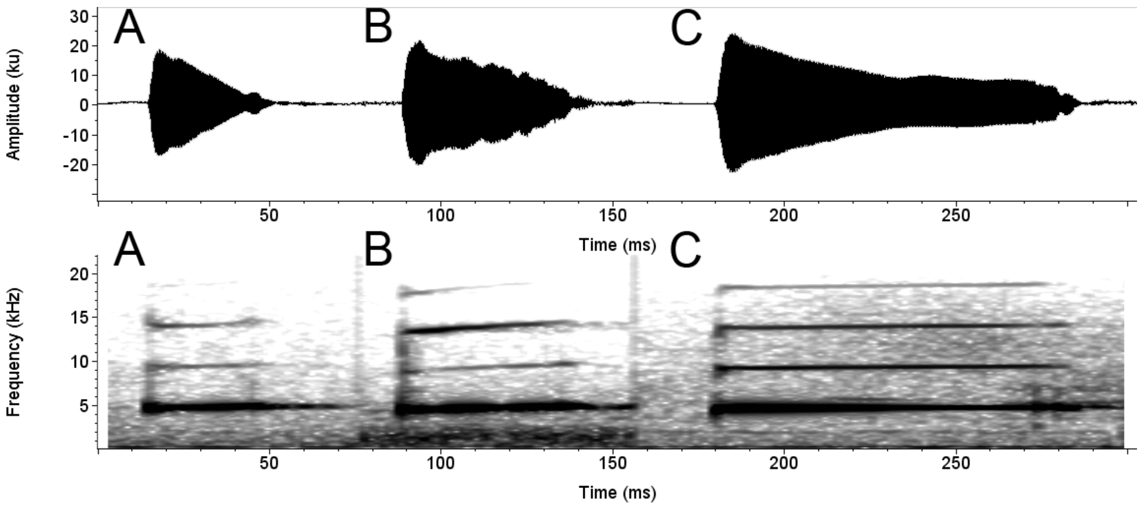


FIGURE 2.

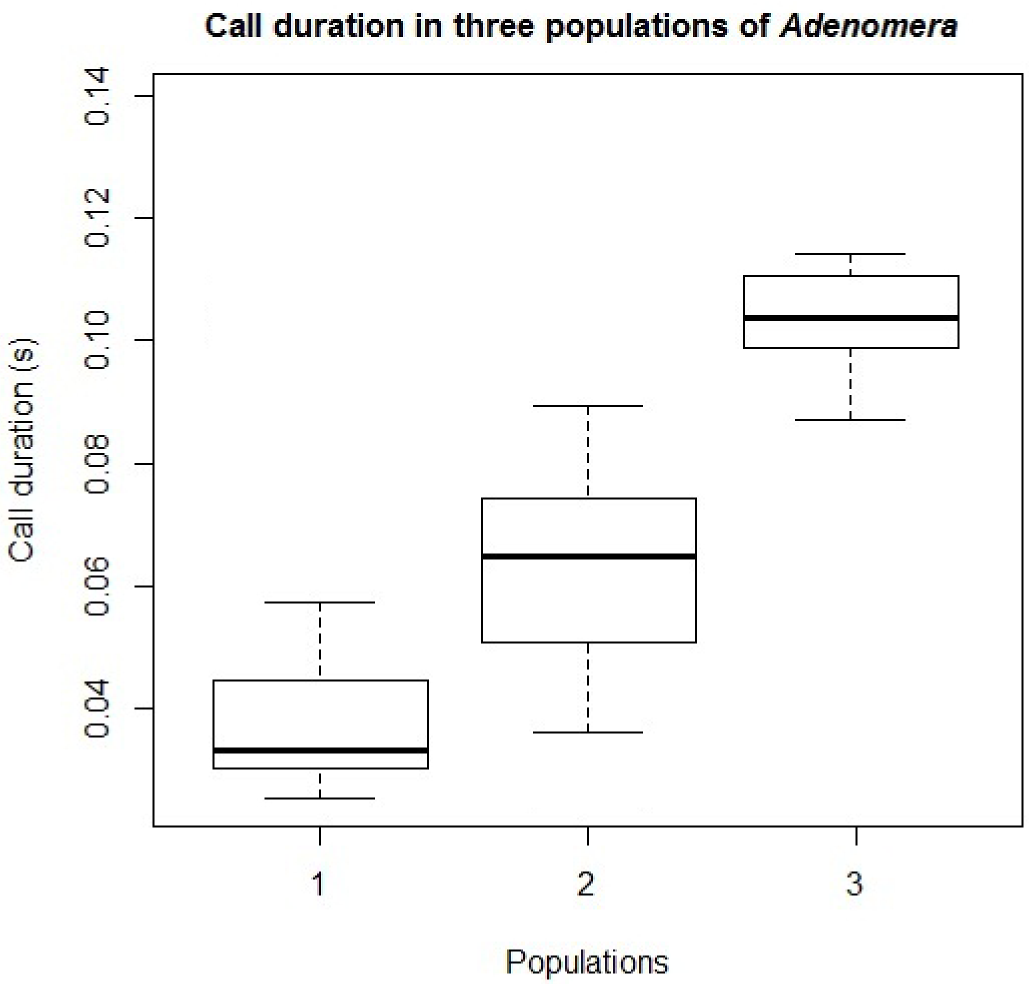


FIGURE 3.

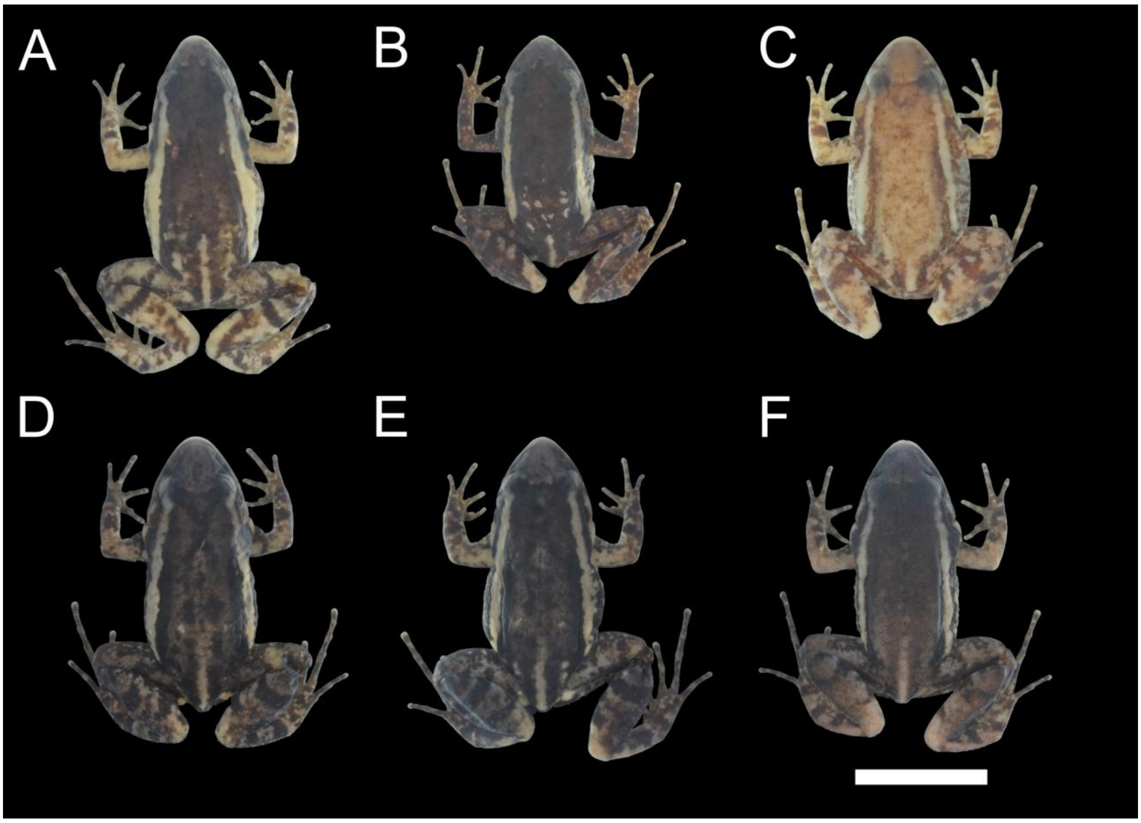
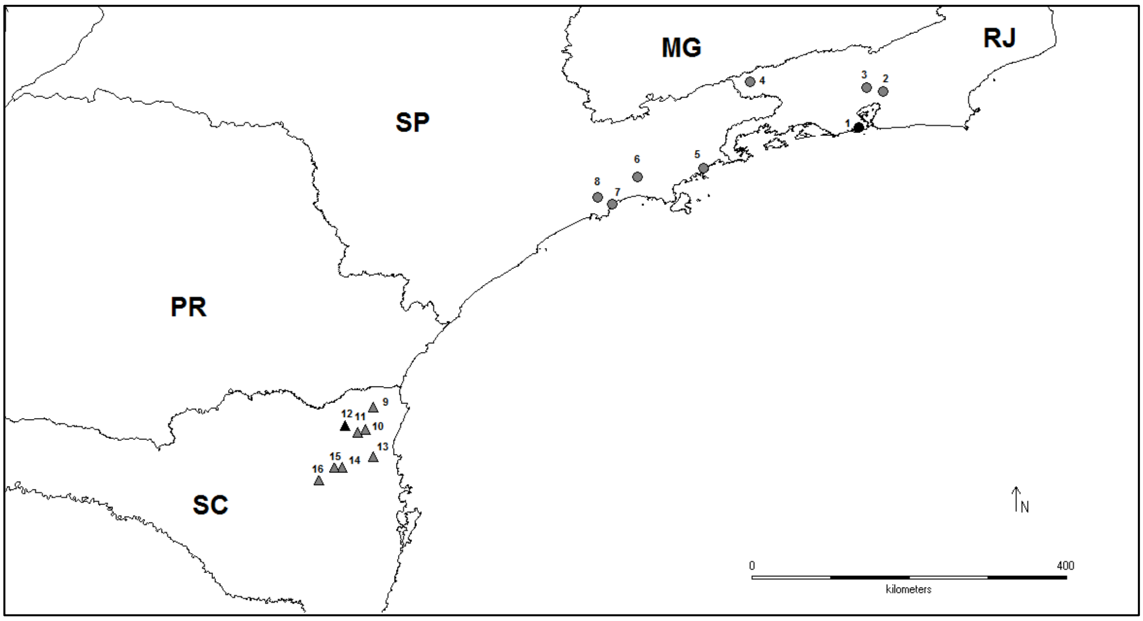


FIGURE 4.



CAPÍTULO II

Use of molecular phylogenetics reveals crypt diversity in the Brazilian Atlantic rainforest frogs of the genus *Adenomera* Steindachner, 1867 (Anura: Leptodactylidae)

USE OF MOLECULAR PHYLOGENETICS REVEALS CRYPT DIVERSITY IN THE BRAZILIAN ATLANTIC RAINFOREST FROGS OF THE GENUS *ADENOMERA* STEINDACHNER, 1867 (ANURA: LEPTODACTYLIDAE)

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1 – INTRODUCTION

Morphologically cryptic species, in other words, two or more distinct species that are classified as a single species (*sensu* Brickford *et al.* 2007), have been a challenge for taxonomists (Brickford *et al.* 2007; Angulo and Icochea, 2010). Tools like bioacoustics and molecular data have become more affordable through the years and has helped to untie specific identity in morphologically cryptic groups, such as amphibians (see Heyer *et al.* 1996; Channing *et al.* 2002; Ron *et al.* 2005; Toledo *et al.* 2007; Angulo and Reichle, 2008; Padial and de la Riva, 2009; Angulo and Icochea, 2010). The Leptodactylid genus *Adenomera*, Steindachner, 1867 is a good example of this situation. It comprises 15 nominal species, more than half of them were described or revalidated in the past eleven years using bioacoustic tools (see Kwet and Angulo, 2002; Almeida and Angulo, 2006; Boistel *et al.* 2006; Kwet, 2007; Angulo and Reichle, 2008, Berneck *et al.* 2008; Kwet *et al.* 2009; Angulo and Icochea, 2010). Studies involving molecular data in attempt to elucidate the taxonomy of the genus have not been made yet.

The Brazilian Atlantic rainforest currently harbors seven nominal species: *Adenomera ajurauna* (Berneck, Costa and Garcia, 2008), *A. araucaria* Kwet and Angulo, 2002, *A. bokermanni* (Heyer, 1973), *A. engelsi* Kwet, Steiner and Zilikens, 2009, *A. marmorata* Steindachner, 1867, *A. nana* (Müller, 1922), and *A. thomei* (Almeida and Angulo, 2006), and this can be an underestimated number of species (Kwet, 2006, 2007).

The first review of the genus *Adenomera* (formerly assigned to the genus *Leptodactylus*) was conducted by Heyer (1973) and at the time only two species were accounted for the Brazilian Atlantic rainforest, *A. bokermanni* and *A. marmorata*. However the two species were extremely widespread, and a few years later it was thought they were complexes of species (Heyer, 1977; 1984). Kwet and Angulo (2002) described *A. araucaria* based mainly on its advertisement call, but they found also some morphological differences. They argued that there were still some undescribed species in southern Brazil. Almeida and Angulo (2006) described *A. thomei* from southeastern Brazil based on its advertisement call and a different reproductive mode, and commented about one undescribed species in the state of Rio de Janeiro. Kwet (2007), after analyzing the bioacoustic data of several *Adenomera* populations over southern Brazil, revalidated *A. nana* and came to the conclusion there were still three undescribed species. Among these species *A. engelsi* were described a couple of years later for the state of Santa Catarina (Kwet, Steiner and Zilikens, 2009). Berneck and colleagues (2008) described the morphologically unique *A. ajurauna*, which occurred sympatrically with *A. marmorata* in the state of São

Paulo. However, despite the efforts in the description of *Adenomera* species in the past few years, there are still species to be formally described.

Studies with molecular data have been useful in order to reveal cryptic diversity in amphibians. For instance, Fouquet *et al.* (2007) found several undescribed species in the *Rhinella margaritifera* and *Scinax ruber* species groups. Padial and de la Riva (2009) described two new *Pristimantis* species from Amazon forest based on mtDNA, morphology and bioacoustics. There are also dramatic examples like the study of Vieites *et al.* (2009). They began their study with 244 described species and came to the conclusion that there were in effect up to 465 species. Therefore molecular studies are of paramount importance in revealing cryptic diversity in amphibians.

Herein, we apply a phylogenetic approach using mitochondrial data (CO1 and Cytb) to try to elucidate the taxonomy of Brazilian Atlantic rainforest *Adenomera* and give comments about formally described and undescribed species.

2 – MATERIALS AND METHODS

2.1 – Taxon sampling

The specimens used in this study were obtained in two ways: (1) through sampling in fieldwork during the years of 2011 and 2012, from which the material were deposited in the Coleção Herpetológica da Universidade Federal de Minas Gerais (UFMG), and (2) through loan of tissue samples from the following collections: Coleção Célio Fernando Baptista Haddad (CFBH), Coleção Herpetológica da Universidade Federal de Minas Gerais (UFMG), Instituto de Investigación Biológica de Paraguay (IIBP), Museu Nacional (MNRJ), and Museu de Zoologia da Universidade de São Paulo (MZUSP).

Tissue samples of all nominal species of *Adenomera* from the Brazilian Atlantic rainforest were taken from thigh muscle or liver. Specimens were sampled from their type locality and from localities of their known geographic distribution (Appendix I). We also sampled tissue of newly discovered populations that were tentatively assigned to one of the Atlantic rainforest *Adenomera* nominal species. With respect to the outgroup, we chose some species of *Adenomera* from the Amazon Forest, some representatives of all *Leptodactylus* species groups (*sensu* Heyer, 1969), and one representative of each other genera within Leptodactylinae, *Hydrolaetare* and *Lythodites* (*sensu* Pyron and Wiens, 2011). We chose also representatives within the two other subfamilies of Leptodactylidae (*sensu* Pyron and Wiens, 2011). *Physalaemus, Engystomops*, *Pseudopaludicola,* and *Pleurodema,* members of the subfamily Leiuperinae and *Paratelmatobius,* a member of the subfamily Paratelmatobinae. Two genera of the family Cycloramphidae (*sensu* Frost, 2013), but *incertae sedis* according to Pyron and Wiens (2011) were used in this study: *Crossodactylodes* and *Rupirana*. We decided to root our tree in the family Centrolenidae, as it is the sister family of Leptodactylidae (Pyron and Wiens, 2011).

For result and discussion we follow the taxonomic arrangement of Pyron and Wiens (2011) and Frost (2013).

2.2 – DNA extraction, amplification and sequencing

For the DNA extraction we used the Quiagen® kit and followed the protocol recommended by the manufacturer. Protocols and standard reagents to the PCR were used to amplify fragments of mitochondrial DNA comprising the regions of the Cytochrome b (735 bp) and Cytochrome c Oxidase I (658 bp).

The used primers were MVZ15 (5' – GAACTAATGGCCCACACWWTACGNAA – 3'; Moritz *et al.* 1992), LeptoR2 (5' – GTGAAGTTRTCYGGGTCYCC – 3'), and LeptoF2 (5' – ATTGCMCAAATYGCYACAGG – 3') (Fouquet, unpublished data) for the Cytb, and LC1490 (5' – GGTCAACAAATCATAAAGATATTGG – 3') and HCO2198 (5' – TAAACTTCAGGGTGACCAAAAAATCA – 3') (Folmer *et al.* 1994) for the COI. The DNA purification and sequencing were realized at MACROGEN Korea.

2.3 – Alignment

The alignment was performed with the implementation Muscle (Edgar, 2004) within the software MEGA version 5 (Tamura *et al.*, 2011). We corrected the alignment manually checking each sequence with the software Bioedit version 7.1.3.0 (Hall, 1999). We obtained a 1393 bp final alignment (cytochrome c oxidase I = 658 bp and Cytochrome b = 735 bp). Bayesian analysis was used to investigate phylogenetic relationships among terminals.

2.4 – Bayesian analyzes

We did two separate Bayesian analyzes. In the first one, we divided the dataset into six partitions, one for each codon position of the citochrome c oxides I (COI) fragment (658/3 bp) and one for each codon position of the Cytochrome b (Cytb) fragment (735/3 bp). In the second one, we divided the dataset into two partitions, one for each gene fragment (COI and Cytb).

We used the software MEGA version 5 (Tamura *et al.*, 2011) to select the substitution model that best fits each of these partitions under the Bayesian information criterion (Schwarz, 1978). The resulting models were employed in a Bayesian analysis with Mr. Bayes version 3.2.1 (Ronquist *et al.* 2012). The analysis consisted of two independent runs of 1.0×10^7 generations, starting with random trees and four Markov chains (one cold), sampled every 1000 generations. The software Tracer version 1.5 (Rambaut and Drummond, 2007) was used to examine likelihood scores, check the convergence of the Markov chains, and effective sample sizes. We discarded 25% of the generations and trees as burn-in. We considered relationships strongly supported when posterior probabilities were equal to or higher than 0.90.

2.5 – New species account

Monophyletic units that could not be assigned to any nominal species were treated as new taxa. The term “cryptic species” is here applicable following Brickford *et al.* (2007) (see introduction).

3 – RESULTS

3.1 – Substitution model and tree choice

The substitution models that best fitted the first analysis, with separate partitions for each codon position were HKY+I for the first and second positions of the codon and GTR+G for the third position of both gene fragments, COI and Cytb. The tests implemented in MEGA version 5 (Tamura *et al.*, 2011) selected a HKY+I as best fitting both gene fragments in the second analysis.

We chose the maximum clade credibility tree originated for the first analysis (Fig. 1) as the best tree because the likelihood ESS score of the first analysis was way higher than that of the second one (1366.77 *versus* 187.67, respectively), and this is the phylogenetic hypothesis discussed hereinafter.

3.2 – Phylogenetic relationships

In total, we analyzed 245 terminals. The COI fragment was sequenced for 224 terminals and the Cytb fragment for 165. 143 terminals had both fragments sequenced.

We recovered a monophyletic *Adenomera* with strong support (100% of posterior probability). Within *Adenomera,* we recovered a Brazilian Atlantic rainforest clade with a relatively strong support (89% of posterior probability). Three major clades were recovered within the Brazilian Atlantic rainforest clade: *Adenomera thomei* clade, Southern Brazil *Adenomera* clade and Southeastern Brazil *Adenomera* clade, all with strong support (100% of posterior probability). The relationships within the Amazon rainforest species and among these species and the Atlantic rainforest species remain ambiguous.

The genus *Leptodactylus* had a strong support (98% of posterior probability), but showed a paraphyletic arrangement regarding the genus *Hydrolaetare*, which was nested within the *Leptodactylus fuscus* species group with strong support (98% of posterior probability).

The subfamily Leptodactylinae was paraphyletic regarding the Paratelmatobiinae subfamily (represented only by a *Paratelmatobius* species) and the genus *Crossodactylodes*, which was the sister group of *Paratelmatobius* with strong support (100% of posterior probability). Leptodactylinae was also polyphyletic regarding *Lithodytes*, which appeared nested in a polytomy close to the root of the tree. But the subfamily Leptodactylinae had a very weak support (69% of posterior probability). The relationships of the Leiuperinae family are extremely ambiguous.

3.3 – Species diversity and new species

Among Amazonian species, *Adenomera andreae* and *Adenomera hylaedactyla* showed paraphyletic and even polyphyletic arrangements. But since the Amazonian species are not the focus of this study, they will not be discussed henceforth.

Among the Brazilian Atlantic rainforest species, all nominal *Adenomera* species were recovered in monophyletic arrangements, and four new species were identified: two in southeastern Brazil and two in southern Brazil. Some of the nominal species had their geographic distribution extended (Fig. 2).

Adenomera ajurauna is described from the municipality of Bertioga, state of São Paulo. We have found *A. ajurauna* in eight more localities within the state of São Paulo (Fig. 2).

Adenomera araucaria is described from São Francisco de Paula, state of Rio Grande do Sul, and represents the southernmost point of the distribution of the genus *Adenomera*. We extended the distribution of the species for São Bento do Sul, state of Santa Catarina (Fig. 2), near the border of the state of Paraná, and it is now the northernmost point of the distribution of the species (26.27º S, 49.38º W).

Adenomera bokermanni has been known for a long time as being complex of species (Heyer, 1977; Heyer 1984). Its huge type series comprises specimens from four Brazilian states. We now restrict the distribution of the species for a few populations in the states of Paraná and Santa Catarina.

Adenomera engelsi is composed of two clades, one of the Santa Catarina Island (municipality of Florianópolis, Santa Catarina, Brazil), and the other from the subjacent localities in the continental Santa Catarina. They do not have substantial differences and may be treated as conspecifics. Kwet *et al. (*2009) mention the lack of dorsolateral stripes as a diagnostic character of *A. engelsi*. We have one recorded specimen (Fig. 3, UFMG10288), from the type locality (Naufragados, Florianópolis, state of Santa Catarina), which is analyzed in this study that have the dorsolateral stripes. It differs from the syntopic *A.*

araucaria by being bigger (SVL 21.48 mm) and by having an unpulsed advertisement call (Taucce, pers. obs.). For these reasons, we propose that the lack of dorsolateral stripes in *A. engelsi* be not anymore a diagnostic character for the species.

The clade marked with an asterisk within the *Adenomera marmorata* clade, which is the sister group of all other *A. marmorata* populations is the population that carries the name *A. trivittata*. *A. trivittata* is currently a junior synonym of *A. marmorata* (Heyer, 1973). Despite being phylogenetically possible to revalidate this taxon according to our data, bioacoustic data shows that the population belongs actually to the *A. marmorata* species. For this and more comments on *A. marmorata* see the first chapter of this dissertation.

Adenomera thomei was described from the state of Espírito Santo, and its distribution was until now restricted to it. Our data show that the species is surprisingly widespread, and it is present in the states of Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo (Fig. 2). Heyer (1973) mentions that he could see some differences (*e. g.* the presence of broad and light dorsolateral stripes in most specimens of southern *A. bokermanni* and the more dorsally directed eyes of northern *A. bokermanni*) between the southern part and the northern part of the distribution of *A. bokermanni*. As *A. thomei* is a *A. bokermanni*–like species (Almeida and Angulo, 2006) and the localities analyzed by Heyer (1973) strongly matches ours, we think that probably most of the specimens analyzed by him are actually *A. thomei*.

4 – DISCUSSION

4.1 – Phylogenetic relationships

Pyron and Wiens (2011) sampled three *Adenomera* species in their phylogenetic study, recovered a monophyletic arrangement and revalidated the genus. Frost (2013) agreed with them and accepted *Adenomera* as a valid genus in his huge amphibian database. Since *Adenomera* is also monophyletic in our study, and it is not nested within any other Leptodactylinae genera, we agree with Pyron and Wiens (2011) and with Frost (2013) and accept *Adenomera* as a valid genus. However, besides the well supported Atlantic forest clade, the relationships among the older divergent lineages could not be recovered.

Adenomera nana was described from the state of Santa Catarina, and was historically related to *A. marmorata* (Cochran, 1955). Heyer (1973) proposed that *A. nana* was a junior synonym of *A. marmorata*. Kwet (2007) revalidated it based on bioacoustics and morphological data. Given its phylogenetic position, we strongly agree with Kwet (2007) and understand that *A. nana* is undoubtedly a valid species.

The genus *Leptodactylus* showed a paraphyletic arrangement regarding *Hydrolaetare*. The genus *Hydrolaetare* was created by Gallardo (1963) to place *Limnomedusa schmidti* Cochran and Groin, 1959, and now comprises three species: *H. caparu* Jansen, Gozales–Álvarez, and Köhler, 2007, *H. dantasi*, (Bokermann, 1959), and *H. schmiditi* (Cochran and Groin, 1959). The diagnostic definition of the genus is based on morphological characters (Gallardo, 1963; Cochran and Goin, 1970; Lynch 1971; Souza and Haddad, 2005) and the phylogenetic position of *Hydrolaetare* was not tested until now.

Bokermann (1959), based on morphological characters, found relationships among *Hydrolaetare* and *Leptodactylus*. Our data agree with this study, but we understand that phylogenetic studies comprising more *Leptodactylus* and *Hydrolaetare* specimens, as well as other taxa that *Hydrolaetare* once belonged (*e. g. Limnomedusa)* are necessary to truly elucidate the taxonomic and the phylogenetic position of the genus.

The genus *Crossodactylodes* showed a sister group relationship with the genus *Paratelmatobius*. The genus *Crossodactylodes* was historically related to a variety of taxa. Lynch (1971) placed it with *Cycloramphus* and *Zachaenus*, within the formerly Leptodactylid subfamily Cycloramphinae, tribe Grypscini. Heyer (1975) related it with *Cycloramphus, Zachaenus* (like Lynch previously did), *Craspedoglossa, Crossodactylus, Hylodes, Megaelosia, Pratelmatobius, Scythrophrys,* and *Thoropa* in the informal group "Grypscines". Dubois (2005) allocated the genus in the family Leptodactylidae, subfamily Cycloramphinae. Pyron and Wiens (2011) placed it as *incertae sedis* as they could not use it in their study. Frost (2013), based on previous morphological studies aforementioned, places *Crossodactylodes* in the family Cycloramphidae. Based on our data, we agree with Heyer (1975) and Dubois (2005) that *Crossodactylodes* is closely related to *Paratelmatobius*. However, the lack of data from other genera historically related to *Crossodactylodes* prevents us to take any taxonomic decisions.

The two mitochondrial genes used in this study (Cytb and COI) are fast evolving and consequently highly variable in amphibians (Graybeal, 1993; Xia *et al.* 2012). For this reason, relationships among the older divergences could not be well recovered and will not be discussed here.

4.2 – Cryptic species diversity in Brazilian Atlantic rainforest

Four candidate new species were accounted, which means more than one half of the recognized species for the Brazilian Atlantic rainforest. This result was somewhat expected, given the recognized cryptic diversity of the genus (De la Riva, 1996; Angulo *et al.* 2003; Kwet, 2007; Ponssa and Heyer, 2007; Kwet *et al.* 2009).

Adenomera sp. 1 was chosen as a candidate new species because despite being closely related to *A. marmorata* it has an overall *A. bokermanni*–like morphology. *Adenomera* sp. 2 is morphologically similar to *A. marmorata,* but it has a different advertisement call (Cassini, pers. obs.). *Adenomera* sp. 3, from the municipality of Morretes, state of Paraná is probably *Adenomera* sp. 3 of Kwet (2007). We are not aware of any morphological traits that differs *Adenomera* sp. 4 from *A. bokermanni*, but if we treated it as such, *A. bokermanni* would show a paraphyletic arrangement. The vocalization of *Adenomera* sp. 4 is not known.

However, it is also known that bioacoustics data are of paramount importance for the taxonomy of the genus *Adenomera*, and has helped in the description and revalidation of species in the past few years (see introduction). Therefore we understand that a “integrative taxonomy” approach (Padial *et al.* 2010), which we consider being nothing more than a well done taxonomy, is necessary to check the taxonomic status of the candidate species in this study and, if confirmed their new species status, describe them.

5 – CONCLUSIONS

The genus *Adenomera* showed a strong supported monophyletic arrangement, and we agree with Pyron and Wiens (2011) and Frost (2013) regarding *Adenomera* as a valid genus. *Leptodactylus* showed a paraphyletic arrangement with *Hydrolaetare* nested within it and *Crossodactylodes* appeared related to *Paratelmatobius*, but the limitation of our data prevents us to take any taxonomic conclusions. More genes and terminals are necessary to elucidate the phylogenetic relationships within the Leptodactylidae family. All nominal species are valid species. There are four candidate new species in this study, but it is necessary an "integrative taxonomy" approach to evaluate the taxonomic status of them.

6 – ACKNOWLEDGEMENTS

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7 – ABSTRACT

Morphologically cryptic species have been a challenge for taxonomists. Bioacoustics and molecular data have become more affordable through the years and are of paramount importance on untying species identity in morphologically cryptic groups, such as Amphibia. The genus Adenomera is a good example of this situation, with more than a half of its nominal species described in the past eleven years based mainly on bioacoustics data. Molecular data are used at the first time to help elucidate the Adenomera taxonomy in the Brazilian Atlantic rainforest. Tissue samples of all nominal species within the Atlantic forest were taken from the type locality and localities along the geographic distribution of the species. We also sampled tissue from newly discovered populations that were tentatively assigned to one of the Atlantic forest described Adenomera species. Bayesian Inference analyzes were performed in the software MRBAYES version 3.2.1. We recovered a monophyletic Adenomera with strong support but a paraphyletic Leptodactylus regarding Hydrolaetare. The family Leptodactylidae showed a paraphyletic arrangement regarding Crossodactylodes and a polyphyletic arrangement regarding Lithodytes. Four candidate Adenomera species were found in the Brazilian Atlantic rainforest.

8 – RESUMO

Espécies morfologicamente crípticas têm se mostrado desafio para os taxonomistas. Dados moleculares e de bioacústica se tornaram mais acessíveis nos últimos anos e são de extrema importância no que concerne a taxonomia de grupos morfologicamente crípticos, como Amphibia. O gênero *Adenomera* é um bom exemplo desta situação, com mais da metade de suas

espécies nominais descrita nos últimos onze anos, com base principalmente em dados de bioacústica. Pela primeira vez dados moleculares são utilizados para ajudar a elucidar a taxonomia de *Adenomera* na Mata Atlântica brasileira. Amostras de tecidos de todas as espécies nominais dentro da Mata Atlântica foram coletadas das localidades tipo e localidades ao longo da distribuição geográfica das espécies. Também foram coletados tecidos de populações recém-descobertas que foram tentativamente atribuídas a uma das espécies de *Adenomera* descritas para a Mata Atlântica. Análises de Inferência Bayesiana foram realizadas no software MrBayes versão 3.2.1. Nós recuperamos um gênero *Adenomera* monofilético, porém o gênero *Leptodactylus* mostrou um arranjo parafilético com relação ao gênero *Hydrolaetare*. A família Leptodactylidae se mostrou parafilética com relação ao gênero *Crossodactylodes* e polifilética com relação ao gênero *Lithodytes*. Quatro candidatas a espécies foram encontradas para as *Adenomera* na Mata Atlântica brasileira.

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APPENDIX I: LIST OF SAMPLED *ADENOMERA* LOCALITIES

Species	Locality	Brazilian state/country
Adenomera ajurauna	PESM Curucutu	São Paulo
Adenomera ajurauna	Piedade	São Paulo
Adenomera ajurauna	Tapiraí	São Paulo
Adenomera ajurauna	São Miguel Arcanjo	São Paulo
Adenomera ajurauna	Paranapiacaba	São Paulo
Adenomera ajurauna	Bertioga	São Paulo
Adenomera ajurauna	Jaceguava	São Paulo
Adenomera ajurauna	Embu	São Paulo
Adenomera ajurauna	Juquitiba	São Paulo
Adenomera andreae	Manaus	Amazonas
Adenomera andreae	Saut Pararé	French Guiana
Adenomera andreae	Brownsberg	Suriname
Adenomera araucaria	Sao Francisco de Paula	Rio Grande do Sul
Adenomera araucaria	Sao Bonifacio	Santa Catarina
Adenomera araucaria	Florianopolis	Santa Catarina
Adenomera araucaria	Bombinhas	Santa Catarina
Adenomera araucaria	Sao Bento do Sul	Santa Catarina
Adenomera araucaria	Vidal Ramos	Santa Catarina
Adenomera bokermanni	Paranagua	Paraná
Adenomera bokermanni	Guaratuba	Paraná
Adenomera bokermanni	Itapoa	Santa Catarina
Adenomera coca	Bateon	Bolívia
Adenomera coca	Muyurina	Bolívia
Adenomera dyptix	Corumbá	Mato Grosso do Sul
Adenomera dyptix	Concepción	Paraguay
Adenomera dyptix	San Bernardino	Paraguay
Adenomera engelsi	Sao Bonifacio	Santa Catarina
Adenomera engelsi	Rancho Queimado	Santa Catarina
Adenomera engelsi	Aguas Mornas	Santa Catarina
Adenomera engelsi	Anitapolis	Santa Catarina
Adenomera engelsi	Anhatomirim	Santa Catarina
Adenomera engelsi	Bombinhas	Santa Catarina
Adenomera engelsi	Itapema	Santa Catarina
Adenomera engelsi	Florianopolis	Santa Catarina
Adenomera heyeri	Tasso Fragoso	Maranhão
Adenomera hylaedactyla	Viçosa do Ceará	Ceará
Adenomera hylaedactyla	Ubajara	Ceará
Adenomera hylaedactyla	Alcântra	Maranhão
Adenomera hylaedactyla	Tasso Fragoso	Maranhão
Adenomera hylaedactyla	Açailândia	Maranhão
Adenomera hylaedactyla	Chapada dos Guimarães	Mato Grosso

Species	Locality	Brazilian state/country
Adenomera hylaedactyla	Cuiabá	Mato Grosso
Adenomera hylaedactyla	Sapezal	Mato Grosso
Adenomera hylaedactyla	Peixe-boi	Pará
Adenomera lutzi	Mount Ayanganna	Guyana
Adenomera lutzi	Potaro river	Guyana
Adenomera lutzi	Mount Wokomung	Guyana
Adenomera marmorata	Ilha Bela	Rio de Janeiro
Adenomera marmorata	Marica	Rio de Janeiro
Adenomera marmorata	Saquarema	Rio de Janeiro
Adenomera marmorata	Cachoeira de Macacu	Rio de Janeiro
Adenomera marmorata	Rio de Janeiro Tijuca	Rio de Janeiro
Adenomera marmorata	Ilha Grande	Rio de Janeiro
Adenomera marmorata	Mangaratiba	Rio de Janeiro
Adenomera marmorata	Petropolis	Rio de Janeiro
Adenomera marmorata	Guapimirim	Rio de Janeiro
Adenomera marmorata	Itatiaia	Rio de Janeiro
Adenomera marmorata	Rio Claro	Rio de Janeiro
Adenomera marmorata	Santos	São Paulo
Adenomera marmorata	Santos	São Paulo
Adenomera marmorata	Sao Vicente	São Paulo
Adenomera marmorata	Itanhanhem	São Paulo
Adenomera marmorata	Alcatrazes	São Paulo
Adenomera marmorata	Barra do Uma	São Paulo
Adenomera marmorata	Paranapiacaba	São Paulo
Adenomera marmorata	Sao Bernardo do Campo	São Paulo
Adenomera marmorata	Bertioga	São Paulo
Adenomera marmorata	Sao Sebastiao	São Paulo
Adenomera marmorata	Mogi das Cruzes	São Paulo
Adenomera marmorata	Biritiba Mirim	São Paulo
Adenomera marmorata	Boraceia	São Paulo
Adenomera marmorata	Cubatao	São Paulo
Adenomera marmorata	Ubatuba	São Paulo
Adenomera marmorata	Santa Branca	São Paulo
Adenomera marmorata	Caieiras	São Paulo
Adenomera marmorata	Santa Isabel	São Paulo
Adenomera marmorata	Santana do Parnaiba	São Paulo
Adenomera martinezi	Montes Claros de Goiás	Goiás
Adenomera martinezi	Novo Progresso	Pará
Adenomera martinezi	Araguacema	Tocantins
Adenomera nana	Guaratuba	Paraná
Adenomera nana	Sao Bento do Sul	Santa Catarina
Adenomera nana	Guamirim	Santa Catarina
Adenomera nana	Blumenau	Santa Catarina

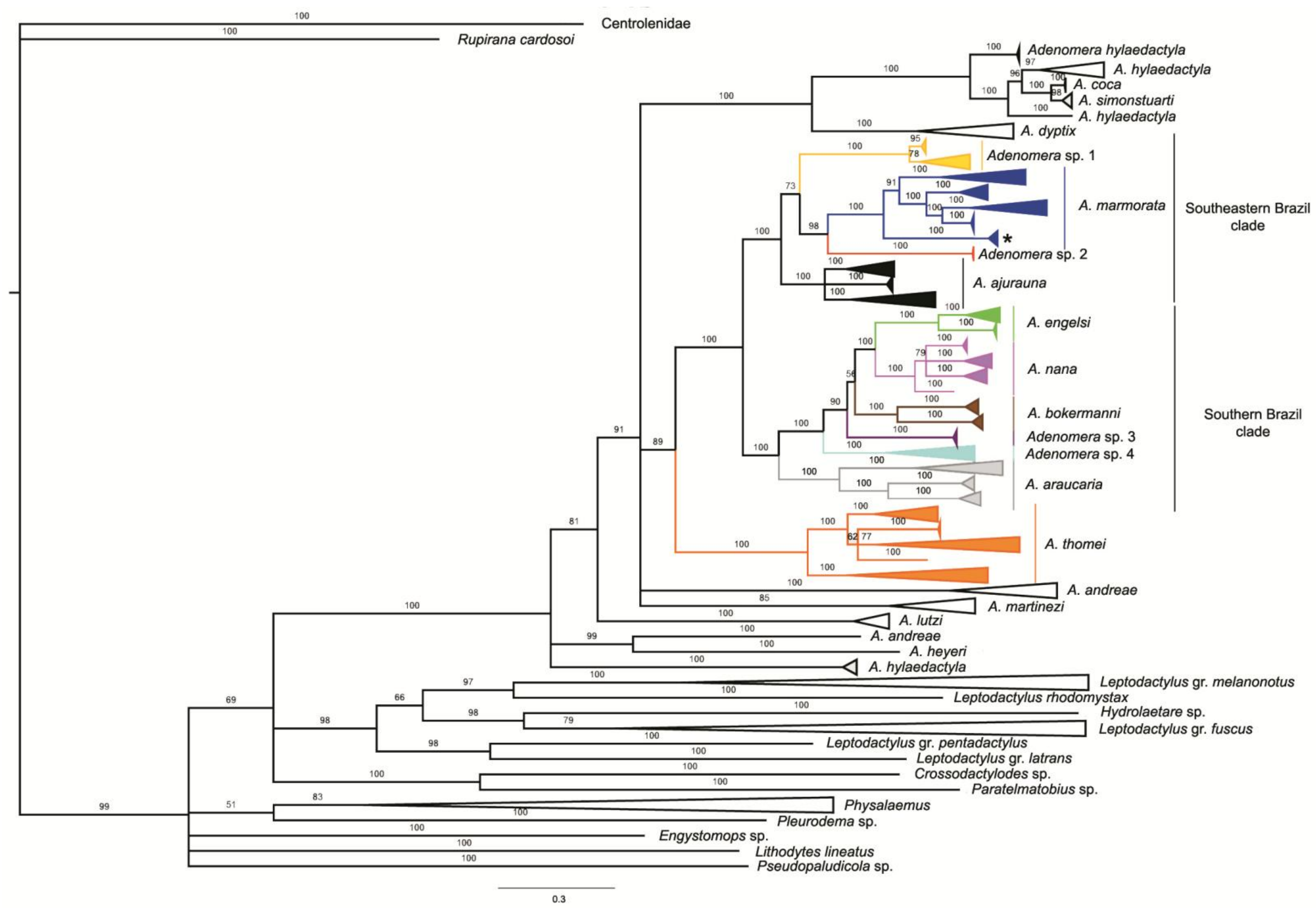
Species	Locality	Brazilian state/country
Adenomera nana	Sao Francisco do Sul	Santa Catarina
Adenomera nana	Joinville	Santa Catarina
Adenomera simonstuarti	Urubamba river basin	Peru
Adenomera sp. 1	Guaruva	Paraná
Adenomera sp. 1	Ribeirao Grande	São Paulo
Adenomera sp. 1	Buri	São Paulo
Adenomera sp. 1	Cananeia	São Paulo
Adenomera sp. 1	Jacupiranga	São Paulo
Adenomera sp. 1	Iporanga	São Paulo
Adenomera sp. 2	Macae	Rio de Janeiro
Adenomera sp. 3	Morretes	Paraná
Adenomera sp. 4	Cananeia	São Paulo
Adenomera sp. 4	Iguape	São Paulo
Adenomera sp. 4	Ilha Comprida	São Paulo
Adenomera thomei	Arataca	Bahia
Adenomera thomei	Unna	Bahia
Adenomera thomei	Linhares	Espírito Santo
Adenomera thomei	Mimoso do Sul	Espírito Santo
Adenomera thomei	Varginha	Minas Gerais
Adenomera thomei	Rio Doce	Minas Gerais
Adenomera thomei	Santa Barbara	Minas Gerais
Adenomera thomei	Serra do Cipo	Minas Gerais
Adenomera thomei	Serra do Caraca	Minas Gerais
Adenomera thomei	Itatiaia	Rio de Janeiro
Adenomera thomei	Macae	Rio de Janeiro
Adenomera thomei	Rio das Ostras	Rio de Janeiro
Adenomera thomei	Mogi das Cruzes	São Paulo
Adenomera thomei	Botucatu	São Paulo
Adenomera thomei	Angatuba	São Paulo
Adenomera thomei	Ipero	São Paulo

Figure 1. Largest posterior probability tree obtained through Bayesian analysis. Numbers over the branches mean posterior probability levels.

Figure 2. Brazilian Atlantic rainforest *Adenomera* distribution map of localities sampled in our analyzes. BA, state of Bahia, ES, state of Espírito Santo, MG, state of Minas Gerais, PR, state of Paraná, RJ, state of Rio de Janeiro, RS, state of Rio Grande do Sul, SC, state of Santa Catarina. Brazil.

Figure 3. Live specimen of *Adenomera engelsi* (UFMG10288) showing the dorsolateral stripes that Kwet *et al.* (2009) mention not to exist for the species.

Figure 1.



Centrolenidae
Rupirana cardosoi
Adenomera hylaedactyla
A. hylaedactyla
A. coca
A. simonstuarti
A. hylaedactyla
A. dyptix
Adenomera sp. 1
A. marmorata
Southeastern Brazil clade
Adenomera sp. 2
A. ajurauna
A. engelsi
A. nana
A. bokermanni
Southern Brazil clade
Adenomera sp. 3
Adenomera sp. 4
A. araucaria
A. thomei
A. andreae
A. martinezi
A. lutzi
A. andreae
A. heyeri
A. hylaedactyla
Leptodactylus gr. melanonotus
Leptodactylus rhodomystax
Hydrolaetare sp.
Leptodactylus gr. fuscus
Leptodactylus gr. pentadactylus
Leptodactylus gr. latrans
Crossodactylodes sp.
Paratelmatobius sp.
Physalaemus
Pleurodema sp.
Engystomops sp.
Lithodytes lineatus
Pseudopaludicola sp.
0.3

Figure 2.

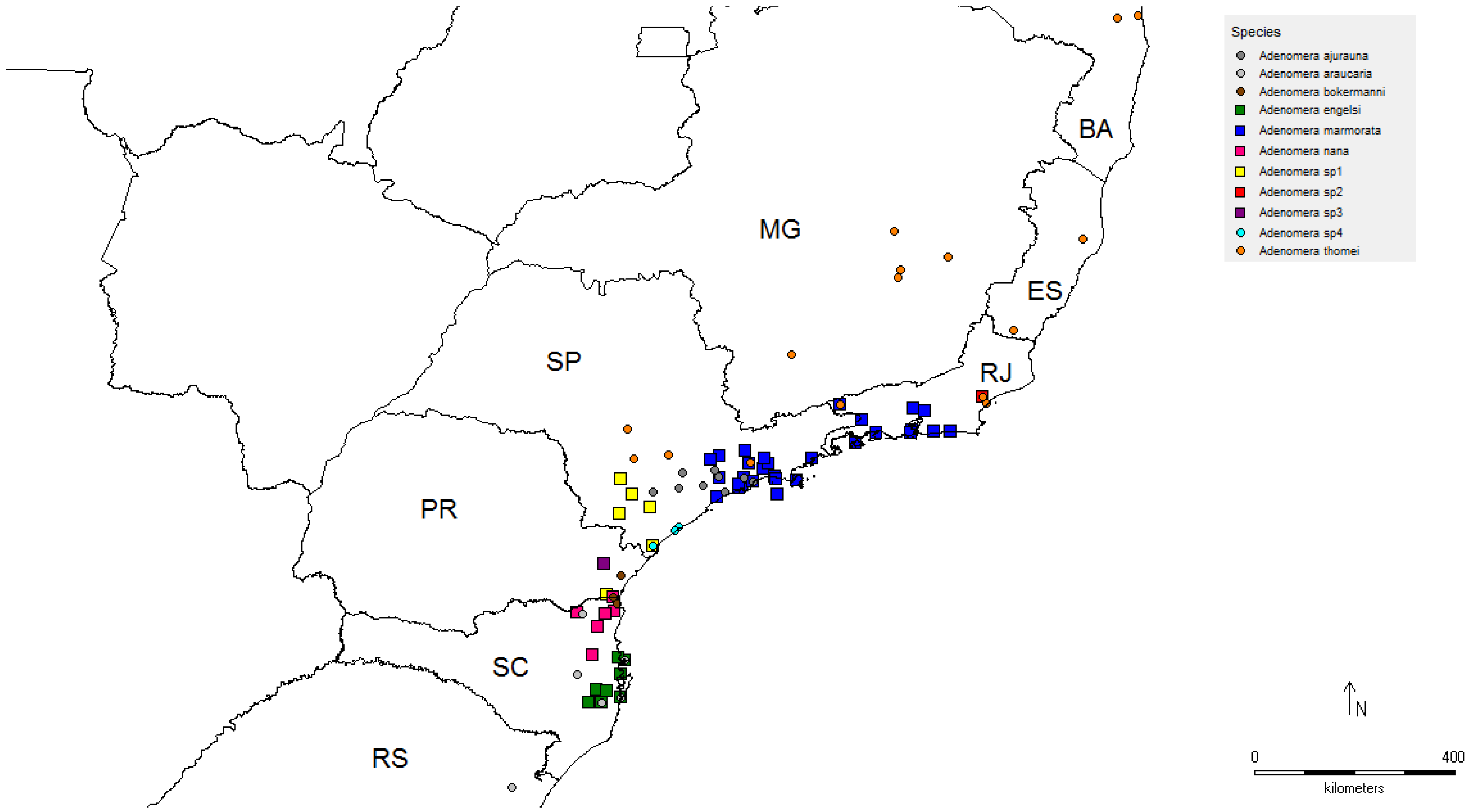

BA
MG
ES
SP
RJ
PR
SC
RS
Species
Adenomera ajurauna
Adenomera araucaria
Adenomera bokermanni
Adenomera engelsi
Adenomera marmorata
Adenomera nana
Adenomera sp1
Adenomera sp2
Adenomera sp3
Adenomera sp4
Adenomera thomei
N
0
400
kilometers

Figure 3.