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Biomoléculas na pele da rã *Lithodytes lineatus* (Anura: Leptodactylidae) mediam a coexistência com formigas cortadeiras do gênero *Atta* (Hymenoptera: Formicidae)

André de Lima Barros

Manaus, Amazonas
Abril, 2016

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Orientadora: Dra. Albertina Pimentel Lima

Coorientador: Dr. Jorge Luís López-Lozano

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Sinopse:

Foi estudado o mecanismo utilizado pela rã *Lithodytes lineatus* para conviver com formigas cortadeiras do gênero *Atta* sem sofrer ataque. Examinamos a hipótese de que a rã apresenta compostos químicos na pele capazes de serem reconhecidos pelas formigas permitindo a coexistência.

Palavras-chave: Reconhecimento químico, *Atta laevigata, Atta sexdens*, *Lithodytes lineatus*.

Dedico tudo o que consegui até aqui, tudo o que sou e tudo que pretendo ser a uma mulher guerreira, que nunca duvidou e nem questionou minhas escolhas acadêmicas.

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RESUMO

Mimetismo e camuflagem químicos são estratégias utilizadas por invertebrados parasitas de insetos sociais, mas o uso desta estratégia por vertebrados para evitar detecção por insetos sociais tem recebido pouca atenção. Aqui examinamos a hipótese de que a rã *Lithodytes lineatus* possui químicos na pele que imitam o reconhecimento químico usado pelas formigas cortadeiras *Atta*. Mostramos que indivíduos de *Lithodytes lineatus* não sofrem ataque pelas formigas *Atta*, enquanto 100% de quatro outras espécies de anuros foram atacados. Adicionalmente, nenhum dos 10 indivíduos do sapo *Rhinella major* embebidos com os extratos de pele da rã *L. lineatus* sofreu ataque, enquanto os controles (embebidos em água ultrapura) foram atacados em cada experimento. Nós demonstramos que a pele da rã *Lithodytes lineatus* possui substâncias químicas que previnem ataque de ambas as espécies de formigas cortadeiras, *Atta laevigata* e *Atta sexdens*.

Palavras-Chave: Reconhecimento químico, formiga, *Atta laevigata*, *Atta sexdens*, *Lithodytes lineatus*.

ABSTRACT

The frog *Lithodytes lineatus* (Anura: Leptodactylidae) uses chemical recognition to live in colonies of leaf-cutting ants of genus *Atta* (Hymenoptera: Formicidae)

Chemically-based mimicry and camouflage is known to be employed by invertebrate parasites of social insect colonies, but the use of this strategy by vertebrates to avoid detection by social insects has received less attention. Here we examine the hypothesis that the frog *Lithodytes lineatus* has skin-chemicals that imitate chemical- recognition used by the leaf-cutting *Atta* ants. We show that individuals of *Lithodytes lineatus* never suffered attack by the leaf-cutting *Atta* ants, while 100% of four other species of anurans were attacked. In addition, none of the 10 individuals of the frog *Rhinella major* coated with skin extracts of the frog *L. lineatus* suffered attack, whereas controls (coated with ultrapure water) were attacked on each occasion. We have demonstrated that the skin of the frog *Lithodytes lineatus* has chemicals that prevent attack by both species of leaf-cutting ants, *Atta laevigata* and *Atta sexdens*.

Keywords: Chemical Recognition, Ant, *Atta laevigata*, *Atta sexdens*, *Lithodytes lineatus.*

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INTRODUÇÃO

As interações entre organismos têm recebido cada vez mais atenção de pesquisadores, e é fato que a maioria das espécies viventes se engaja em pelo menos uma interação interespecífica (positivas, negativas ou neutras) ao longo de seu ciclo de vida (Brostein *et al.*, 2006; Dáttilo *et. al.*, 2009). As associações entre espécies podem ocorrer em prol de um benefício mútuo ou individual e são comuns entre invertebrados e plantas na relação parasita - hospedeiro (Ricklefs, 2010; Menzel *et al.*, 2014).

Vários invertebrados desenvolveram mecanismos para conviver com outras espécies de invertebrados, em relações mutualísticas ou parasíticas (Prestes e Cunha, 2012; Menzel *et al.,* 2014). Em relações parasíticas, a escolha do mecanismo utilizado pelo invasor, favorece a entrada nos ninhos e minimiza possíveis respostas negativas pelos residentes (Lenoir *et al.,* 2001; Tsuneoka e Akino, 2008; Tsuneoka e Akino, 2012).

Em insetos parasitas de insetos sociais (formigas, cupins, vespas e abelhas), um dos mecanismos mais eficientes é a utilização de químicos que "quebrem" o sistema de reconhecimento e comunicação da colônia, realizados por partículas químicas presentes nos indivíduos (espécie-específico) ou no ninho (colônia-específico). Qualquer fator que altere o reconhecimento dos habitantes da colônia pode causar fortes impactos negativos aos residentes (Wilson, 1961; Hölldobler e Wilson, 1986; Breed *et al.,* 1992; Meer e Morel, 1998; Oldam *et al.,* 2002; Hojo *et al.,* 2009; Martin *et al.,* 2008).

Associações entre invertebrados e algumas espécies de anuros tem sido raramente registradas. Existe um grande número de predadores de anuros, sejam vertebrados ou invertebrados. Dessa forma, para evitar a predação, esses indivíduos desenvolveram uma grande variedade de estratégias de defesa (Haddad *et al.,* 2009; Toledo *et al.,* 2011; Lima *et al.,* 2012).

Alguns estudos têm reportado a associação entre sapos microhilídeos e aranhas thereposides (Karunarathna e Amarasinghe, 2009; Dundee *et al.*, 2012). Esse comportamento é considerado bastante incomum, uma vez que aranhas são predadores vorazes de anuros (Menin *et al.,* 2005; Haddad *et al*., 2009; Pombal Jr, 2007; Nascimento *et al*., 2013). O sapo *Chiasmocleis ventrimaculata*, por exemplo, vive em uma relação comensal com aranhas *Xenesthis immanis.* Ambas as espécies dividem uma mesma toca e apesar de *X. immanis* se alimentar de anuros, o sapo não é atacado (Cocroft e Hambler, 1989). A utilização de tocas de aranhas como abrigo confere proteção para o sapo. O mesmo comportamento foi observado entre o anuro *Kaloula tapobranica* e a aranha *Poecilotheria hanumavilasumica* (Siliwal e Ravichandran, 2008). Em ambos os trabalhos, os resultados sugerem que características químicas sejam responsáveis pelo reconhecimento dos sapos pelas aranhas.

Outro tipo de associação, bastante incomum, ocorre entre anuros e formigas na África. Os sapos *Phrynomantis microps* e *Kassina fusca* foram observados associando-se às formigas *Paltothyreus tarsatus* e *Megaponera foetens,* utilizando os ninhos dessas formigas como abrigo. O mais intrigante é que essas formigas são carnívoras e muito agressivas (Déjean *et al.,* 1993), porém estes indivíduos permanecem nos ninhos sem serem atacados (Rödel e Braün, 1999). Experimentos realizados pelos autores sugerem que o comportamento tolerante das formigas é devido a químicos presentes na pele do sapo. No entanto, o mecanismo utilizado ainda permanecia desconhecido.

Anos mais tarde, Rödel *et al.* (2013), continuaram os estudos utilizando *P. microps.* O objetivo do estudo foi descobrir qual o mecanismo utilizado pelo sapo para coexistir com as formigas africanas *Paltothyreus tarsatus* sem sofrer ataque. Os resultados mostraram que o sapo utiliza estratégias contrárias aos anuros comensais. Ao invés de apresentar químicas que favorecessem o reconhecimento pelas parceiras de interação, *P. microps* anula a percepção das formigas permanecendo camuflado na colônia e evitando o ataque por parte dos residentes. Através de análises de separação e identificação de substâncias, como cromatografia e de espectrometria de massa, e a realização de experimentos utilizando a secreção de *P. microps,* os autores mostraram que dois peptídeos presentes na secreção do anuro são provavelmente responsáveis por manter o sapo imperceptível nos ninhos.

Nas Américas, a única associação entre formigas e anuros conhecida, até o presente momento, ocorre entre a rã *Lithodytes lineatus* (Leptdactylidae) e formigas saúvas *Atta* (Hymenoptera, Formicidae). Apesar da população tradicional da Amazônia já ouvir adultos vocalizando no interior dos ninhos, o qual acreditavam ser a "mãe do sauveiro" responsável por emitir o som, a associação de *L. lineatus* com *Atta cephalotes* foi descrita pela primeira vez por Schlüter (1980), onde um indivíduo de *L. lineatus* foi ouvido vocalizando no interior do ninho das formigas. Outras observações da associação entre a rã e as saúvas *Atta cephalotes* foram realizadas por Schlüter e Rëgos (1981). Estes autores sugeriram que se trata de uma relação mutualística entre as formigas e a rã. Posteriormente, outros estudos foram sendo publicados reforçando a existência desta associação (De La Riva, 1993; Lamar e Wild, 1995).

Schlüter *et al* (2009), reportaram a presença de girinos no interior de ninhos ativos de *Atta cephalotes.* Esse achado mostra que, ao contrário dos sapos africanos, a rã utiliza os ninhos das saúvas não apenas como abrigo, mas também como sitio reprodutivo. Os autores apontam algumas possíveis explicações para o estabelecimento da rã nos ninhos como a proteção contra predadores para os seus ovos e larvas e o microclima estável e maior umidade dentro dos ninhos.

Apesar dos vários trabalhos reportando a associação entre estes organismos, o mecanismo utilizado pela rã para viver nos ninhos de formigas sem sofrer ataque ainda não havia sido estudado.

OBJETIVO GERAL

Testar se biomoléculas presentes na pele da rã *Lithodytes lineatus* são responsáveis por permitir a coexistência com formigas cortadeiras do gênero *Atta*.

OBJETIVOS ESPECÍFICOS

a) Testar se a coexistência entre a rã *L. lineatus* e formigas do gênero *Atta* é espécie – específica.

b) Avaliar se o mecanismo utilizado por *L. lineatus* para conviver com as saúvas *A. sexdens* e *A. laevigata* é mediado por substâncias na pele.

ARTIGO

Barros, A. L.; López-Lozano, J. L.; Lima, A. P. **The frog *Lithodytes lineatus* (Anura: Leptodactylidae) uses chemical recognition to live in colonies of leaf-cutting ants of the genus *Atta* (Hymenoptera: Formicidae)**

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The frog *Lithodytes lineatus* (Anura: Leptodactylidae) uses chemical recognition to live in colonies of leaf-cutting ants of the genus *Atta* (Hymenoptera: Formicidae)

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Abstract

Background: Chemically-based mimicry and camouflage is known to be employed by invertebrate parasites of social insect colonies, but the use of this strategy by vertebrates to avoid detection by social insects has received less attention. Here we examine the hypothesis that the frog *Lithodytes lineatus* has skin-chemicals that imitate chemical-recognition used by the leaf-cutting *Atta* ants.

Results: We show that individuals of *Lithodytes lineatus* never suffered attack by the leaf-cutting ants of the genus *Atta*, while 100% of four other species of anurans were attacked. In addition, none of the 10 individuals of the frog *Rhinella major* coated with skin extracts of the frog *L. lineatus* suffered attack, whereas controls (coated with ultrapure water) were attacked on each occasion.

Conclusion: We have demonstrated that the skin of the frog *Lithodytes lineatus* has chemicals that prevent attack by both species of leaf-cutting ants, *Atta laevigata* and *Atta sexdens*.

Keywords: Chemical Recognition, Ant, *Atta laevigata*, *Atta sexdens*, *Lithodytes lineatus.*

Background

Communication between colony-members of social insects, or parasites of these colonies, is often chemically based. In the case of parasites, the chemicals serve to mimic or mask their presence within the colony [1-5].

Frogs have many chemical defenses in their skins, generally used to protect against fungi, bacteria and predators [6,7] However, the role of skin-derived chemicals in interactions between frogs and invertebrates has been rarely recorded, exceptions being cases of mutualism and/or commensal relationships involving some species of microhylid frogs and theraphosid spiders [8-12]. Similarly, associations between frogs and ants are poorly reported in the literature, being known only for the anuran species *Kassina fusca*, *Kassina senegalensis* and *Phrynomantis microps* living in nests of ants in African savanna [13,14].

In the Americas, the only known association between ants and frogs occurs between the frog *Lithodytes lineatus* and ants *Atta* [15-17]. *Lithodytes lineatus* not only uses the nests as shelter, but also uses the colony as a breeding site, building "nests within the nests" [18].

In Africa, peptides present in the skin secretions of the frog *P. microps* are responsible for inhibiting the attack of the ant *Paltothyreus tarsatus* [19]. In the Amazon, the mechanisms used by *L. lineatus* to live with *Atta* ants have not yet been studied. However, it is known that the colonies of *Atta* use chemical substances for recognition and communication [20-22].

We hypothesized that the frog *L. lineatus* produces chemicals on the skin that are recognized by *Atta* ants as a cue for preventing attack. To test this hypothesis, we conducted two field bioassays. In the first treatment, individuals of *L. lineatus* and two genetically (*Adenomera*) and phenotypically similar species of anurans (*Amereega picta* and *Allobates femoralis*) were exposed to *Atta* ants. In the second treatment, *Rhinella* frogs coated with the substance extracted from the skin of *L. lineatus* and coated with ultrapure water (controls) were presented to *Atta* ants to evaluate whether the chemicals that prevent attacks are present in the skin of *L. lineatus*.

Natural history of organisms used in this study

Lithodytes lineatus is a species of frog widely distributed in the Amazon and Orinoco river basins of northern South America. It is known to be associated with leaf-cutting *Atta* ants. *L. lineatus* and is the only species of frog known to breed in nests of ants, where males call [15-18]. Like many leptodactylids, *L. lineatus* lays its eggs out of water in foam nests and tadpoles develop until metamorphosis in the anthill [15-18].

Lithodytes lineatus was considered by several authors to mimic poisonous and aposematic frogs of the genus *Ameerega* and *Allobates* [16, 17]. Prates *et al*. [23], investigated the possible mimicry between *L. lineatus* and *Ameerega picta* in terms of the morphology, distribution of poison glands, skin compounds secreted and behaviour of both species. Their results suggest that *L. lineatus* is as toxic or unpalatable to potential predators as *A. picta*.

Atta spp.

Leaf-cutting *Atta* ants are restricted to the Neotropics, and there are three described species in Amazonia [24]. *Atta* ants feed mostly on a fungal symbiont cultivated within the nests, and do not require external prey organisms [25]. Communication and recognition is given through chemical signals and other ant species or conspecifics that do not have chemical odors similar to those of colony members are attacked by residents [21, 22].

Results

Chemical recognition test using *Lithodytes lineatus* and four other species of anurans

None of the ten individuals of *L. lineatus* placed in the *Atta* nest were attacked, whereas 10 individuals of the genus *Adenomera* suffered an average of 20.1 attacks (Maximum = 50 and Minimum = 9). Individuals of the phenotypically similar species [*Allobates femoralis* (N = 4) and *Ameerega picta* (N = 6)] suffered an average of 20.7 attacks (Maximum = 52 and Minimum = 12) times (Fig. 1). There was a highly significantly lower attack rate (Kruskal-Wallis Test: $H = 20.011$, $df = 2$, $P < 0.001$) on *L. lineatus* than on the four other species tested.

The time between the arrivals of the individual in the colony and attack varied among the species. There was a significant difference (Kruskal-Wallis Test: $H = 13.028$, $df = 2$, $P = 0.001$) in the time to the ant attack on *L. lineatus* and the four species tested.

Individuals of *A. picta* were attacked on average of 0.36 min. (Maximum = 0.71, Minimum = 0.13 min.; N = 6) after the first contact, followed by the species of *Adenomera* (mean = 1.19 minutes; Maximum = 4.37, Minimum = 0.05 min.; N = 10) and *A. femoralis* (mean = 3.78 minutes; Maximum = 8.34, Minimum = 0.78 min; N = 4) (Fig. 2).

Chemical recognition test using skin extracts of *L. lineatus*

No individual of *Rhinella major* coated with skin extracts of *L. lineatus* was attacked by ants (Fig. 3), but all individuals coated only with ultrapure water were attacked. On average, 14.3 ants (Maximum = 46 and Minimum = 5) bit the frogs treated with ultrapure water (Fig. 3). There was a highly significant difference (Kruskal-Wallis Test: H = 16.365, df = 1, P < 0.001) in ant response between treatments.

The ant attacks occurred an average of 0.35 seconds (Max = 0.56 and Min = 0.05 min.) after the first contact with individuals of *R. major* coated in ultrapure water (Fig. 4). There was a highly significant difference (Kruskal-Wallis Test: H = 16.323, df = 1, P < 0.001) in time to attack by the ants among the frogs. Some individuals coated with skin extracts of *L. lineatus* were bitten only when they jumped on the ants, but they were released shortly after the bite.

The forebody of one individual of *R. major* were coated with skin extracts of *L. lineatus* and their hind legs were coated with ultrapure water. The ants walked over the region coated with skin extracts of *L. lineatus*, but did not bite. However, the hind legs were bitten by ants at first contact.

The ants did not attack individuals of *R. major* coated with skin extracts from either male or female *L. lineatus*, demonstrating that both sexes have compounds in the skin that inhibit attack by the ants.

Discussion

Coexistence of frogs and ants is unusual and the mechanisms that allow this poorly known [19]. These authors suggested that the coexistence of the frog *Phrynomantis microps* with the ant *Paltothyreus tarsatus* is possible due to two peptides produced by the frog.

We showed that species of frogs that do not usually associate with ants were attacked aggressively. Neither the phylogenetic proximity of *Adenomera* to *L. lineatus*, nor the phenotypic similarity of *Allobates femoralis* and *Ameerega picta* prevented the attack by ants. All four anurans species tested, which are not associated with the ants tried to escape by jumping and/or climbing the glass vessel wall after contact and attack by the ants. In contrast, no escape attempts were made by *L. lineatus* during the ten-minute experiment, even when the frogs were contacted by ants.

Substances on the skin of *L. lineatus* prevented ant attacks on the frog *Rhinella major*. Therefore, we suggest that the frog *L. lineatus* has chemical compounds on the skin that can be recognized by the soldiers of *Atta laevigata* e *Atta sexdens*. Analysis of the chemicals used by *L. lineatus* may shed light on the chemical mechanisms used by *Atta* species to detect intruders, which are poorly understood [26, 21, 22].

Our two experimental procedures clearly demonstrate that there is a chemical compound on the skin of *L. lineatus* that prevents attack by ants of the species *A. laevigata* and *A. sexdens*. We also showed that neither *L. lineatus*, nor individuals of *R. major* coated with skin extracts from individuals of *L. lineatus* were attacked by the *A. sexdens* soldiers.

There are several reasons why it may be advantageous for the frog to live within the nests of leaf-cutting ants. Some authors have suggested that the anthill´s stable microclimate and higher humidity than the external environment, which are important features for the reproduction of frogs, may be favorable to the frog and their eggs [16, 18].

Amazon has among the highest diversities of anuran reproductive strategies. Due to this high diversity, Magnusson and Hero [27] suggested that the ovipostion outside the aquatic environment is a strategy used by several Amazonian anuran species to avoid predation. However, many terrestrial eggs suffer high predation from invertebrates, such as spiders [28], flies [29], beetles and wasps [30], as well as vertebrates, such as snakes [31] and turtles [32].

Leaf cutter ants aggressively defend their nests against intruders, so we suggest that the ants may help defend the eggs and larvae of *L. lineatus*, which builds its foam nests inside the ant galleries.

Conclusions

We have demonstrated that the leaf-cutting ants of the genus *Atta* do not attack the frog *Lithodytes lineatus*, but aggressively attack other species of anurans. Individuals of *Rhinella major* coated with skin extracts of *L. lineatus* are not attacked when exposed to ants, but individuals of the same species coated with ultrapure water were attacked. Our results support the hypothesis that *L. lineatus* has chemical compounds on the skin that are recognized by ants of the genus *Atta* and these allow for coexistence.

Experimental Procedures

Animals

Field work was carried out in three areas: 1- Reserva Adolpho Ducke (Ducke), Manaus City, Amazonas State; 2- The left bank of the Jaci Paraná River (Jaci Paraná), Rondônia State; and 3- The left bank of the Madeira River (Madeira), Rondônia State. Individuals of *L. lineatus* were found through vocalization. Two individuals were collected in Ducke, two on Jaci Paraná and six individuals on Madeira. Five individuals were captured in nests of *Atta laevigata* and five individuals in the nests of *Atta sexdens*. For controls, *Ameerega picta* individuals (N = 6), *Allobates femoralis* (N = 4) and a species of the genus *Adenomera* (N = 10) were collected in the same areas as the *L. lineatus*.

Chemical recognition test using *Lithodytes lineatus* and four other species of anurans

Frequency of attack by ants on *L. lineatus* was used as an index of the presence of skin chemicals that inhibit aggression. Two species of *Adenomera* spp., a sister clade to that containing the genus *Lithodytes* [33], and *A. femoralis* and *A. picta*, which are phenotypically similar to *L. lineatus,* were used as controls.

Species of *Atta* feed on a fungus grown inside the nests [25] and attack other organisms only to defend the colony. For this reason, potential predators are only attacked when trying to enter the colony, which led us to carry out the experiments at the entrances of the ant nests. Experiments were conducted in *Atta sexdens* colonies, located in Jaci Paraná (N = 2) and in Ducke (N = 2), and in three colonies of *Atta laevigata* located in Madeira. Experimental frogs were placed in opened topped clear glass containers and these were put at the entrances to ant nests.

The experiments consisted of three treatments: 1) individuals of *Adenomera* spp. positioned near the entrance to nests; 2) individuals of *A. femoralis* or *A. picta* positioned near the entrances to nests; and 3) individuals of *L. lineatus* positioned near entrances to nests. In each experiment, the sequence of the anuran species was switched to demonstrate that the order of presentation did not confound the results. In the nests in which more than one experiment was carried out, a different ant nest was used for each experiment. Each frog was used only once, and the experiment had a maximum duration of ten minutes. At the end of that time, the frogs were removed and the number of ants attached to the skin was counted, except in cases where the frogs body was covered by many ants and it was immobilized, in which case the experiment was terminated to prevent the death of frogs. The response time (attack) of ants was defined as the time from the beginning of the experiment until an ant bit and remained attached to the frog (see Additional file 1).

Obtaining the skin extracts of *L. lineatus*

Frogs were killed by overdose with an anesthetic (ointment with 2% benzocaine) applied only within the mouth so that the skin was not contaminated. Following death, the skin was removed for analysis in the laboratory of molecular toxinology of Centro de Ofidismo Prof. Dr. Paulo Bührnheim of Fundação de Medicina Tropical – HVD, Manaus, Amazonas, Brazil.

Skin extracts of males and females were obtained separately, from immersed skins in 1.5 ml of methanol at room temperature. After 72 hours, the methanol was evaporated using a vacuum concentration system at 30°C. Subsequently, 10 mg of frog-skin extract was dissolved in 1.5ml of ultrapure water.

Chemical recognition test using skin extracts of *L. lineatus*

We use the methodology adapted from Rödel *et al*. (2013) to test if the chemical compounds present in the skin of *L. lineatus* were responsible for avoiding ant attack. We used individuals of the frog *R. major* as experimental subjects. *Rhinella major* is an abundant frog in the region and its dry tegument absorbs liquids quickly.

The experiments had two treatments: 1) ten individuals of *R. major* coated with ultrapure water and 2) ten individuals of *R. major* coated with *L. lineatus* skin extracts. Five individuals were coated with skin extracts of males and five individuals were coated with skin extracts of females to test whether there was an effect of sex on the attack by ants.

We use the skin extracts of individuals collected in the colonies of both species of *Atta*. In each treatment, individuals of *R. major* were placed near the entrances of *Atta* nests. Frogs were tethered by a cotton thread tied around their waists. Each frog was used only once. At the end of five minutes, the number of ants stuck to the skin of the frog was registered.

The response time (attack) of ants was defined as the time from the beginning of the experiment until an ant bit and remained attached to the frog (see Additional file 2).

Statistical analyses

To test the differences in the response of the ants (attack/non attack) in both experiments, we use the Kruskal-Wallis Test (nonparametric test). The analyses were performed using R ver. 3.2.3 statistical software.

Additional files

Additional file 1: Experiment 1 (Movie/ MP4 19.5 MB)

Additional file 2: Experiment 2 (Movie/ MP4 10.7 MB)

Ethics committee

The permissions that are required for the realization of experiments in this study were obtained through the Commission of Ethics in the Use of Animals (CEUA) of the Instituto Nacional de Pesquisas da Amazônia (INPA/CEUA, Protocol: 034/2014).

All individuals of *Adenomera*, *Allobates femoralis*, *Ameerega picta* and *Rhinella major* used in the experiments were released after the experiment. They had no visible injuries and showed no signs of distress. These species naturally interact frequently with *Atta* species in the wild.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ALB, APL and JLLL designed the methodology of the study. ALB and APL wrote the manuscript together. ALB collected the anuran species. APL coordinated the activities of this study. ALB and JLLL obtained skin extracts of *L. lineatus*. All authors read and approved the final manuscript.

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FIGURES LIST

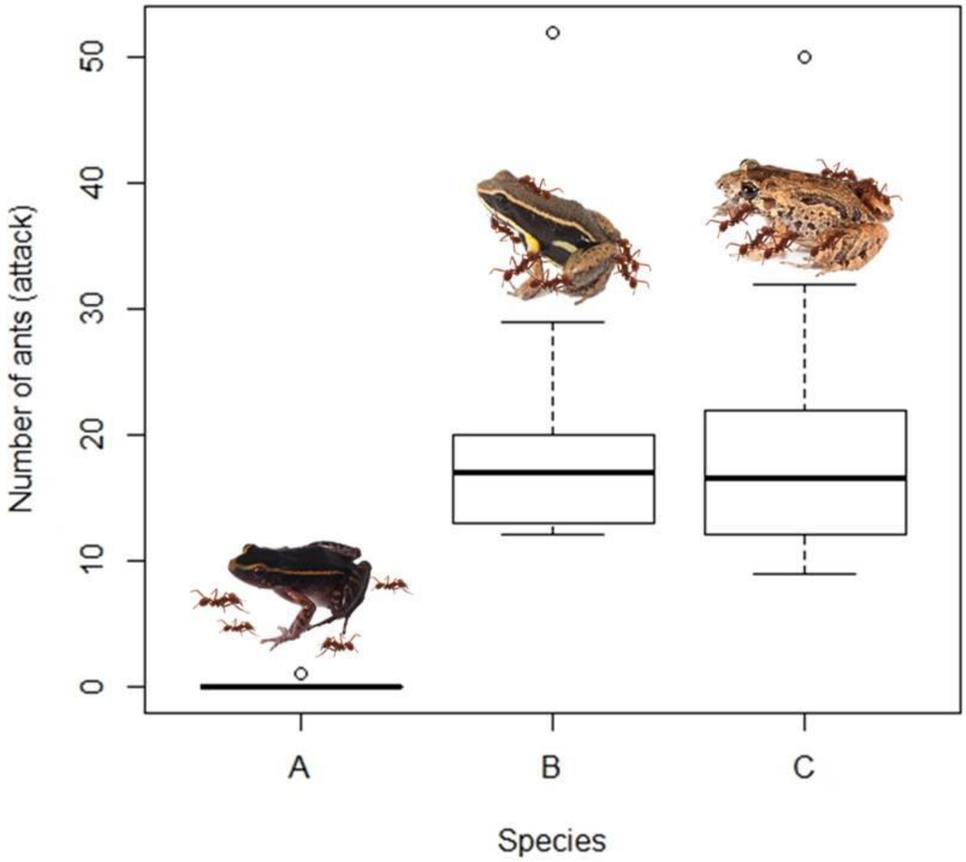

Fig. 1. Response (attack) of the ants *Atta* spp., when exposed to different species of anurans. **A**, corresponds to *Lithodytes lineatus* (partner relationship; N = 10); **B**, corresponds to *Ameerega picta* (N = 6) and *Allobates femoralis* (N = 4); and **C**, corresponds to species of the genus *Adenomera* (N = 10).

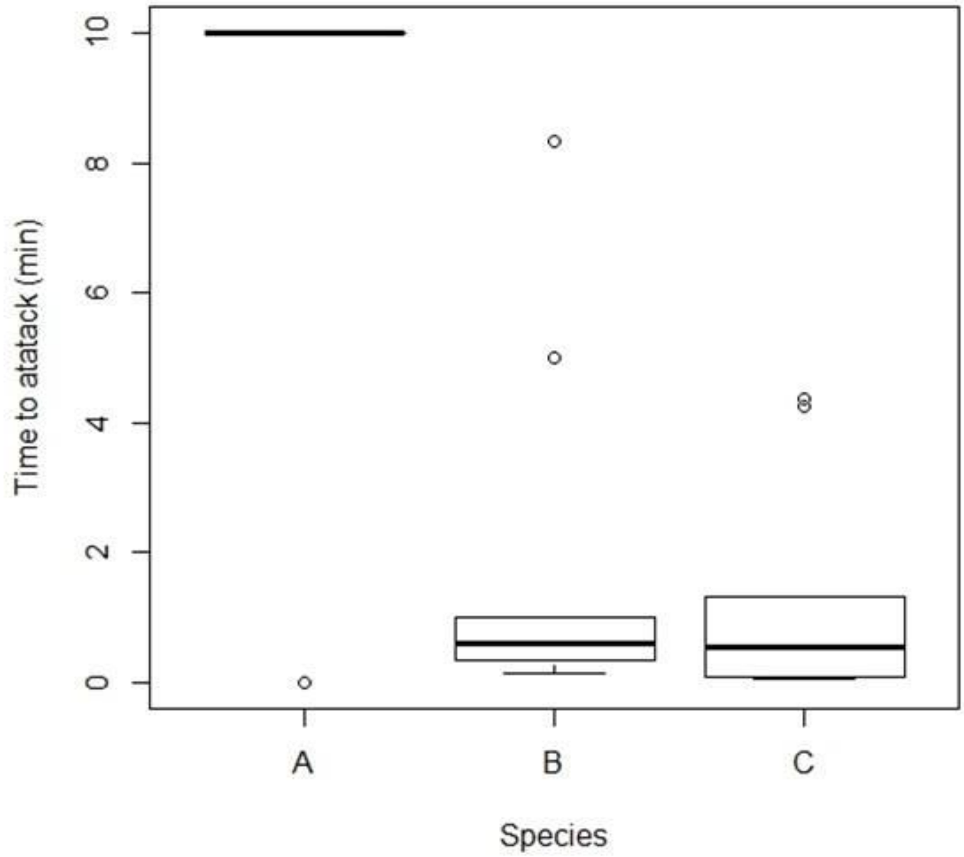

Fig. 2. Response time of the ants *Atta* spp., when exposed to different species of anurans. **A**, corresponds to *Lithodytes lineatus* (partner relationship, N = 10); **B**, corresponds to *Ameerega picta* (N = 6) and *Allobates femoralis* (N = 4); and **C**, corresponds to species of the genus *Adenomera* (N = 10).

FIGURES LIST (Continuação)

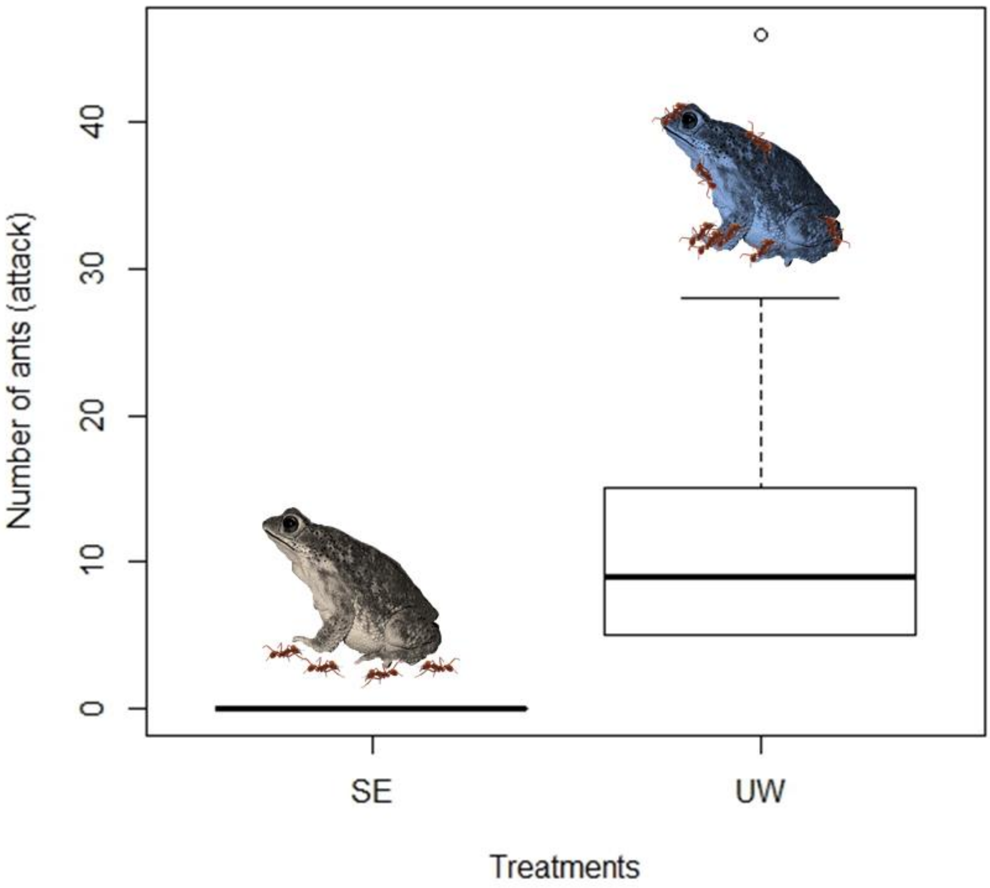

Fig. 3. Response (attack) of the ants when exposed to individuals of *Rhinella major* coated with skin extracts (SE; N = 10) of the frog and coated with ultrapure water (UW; N = 10).

FIGURES LIST (Continuação)

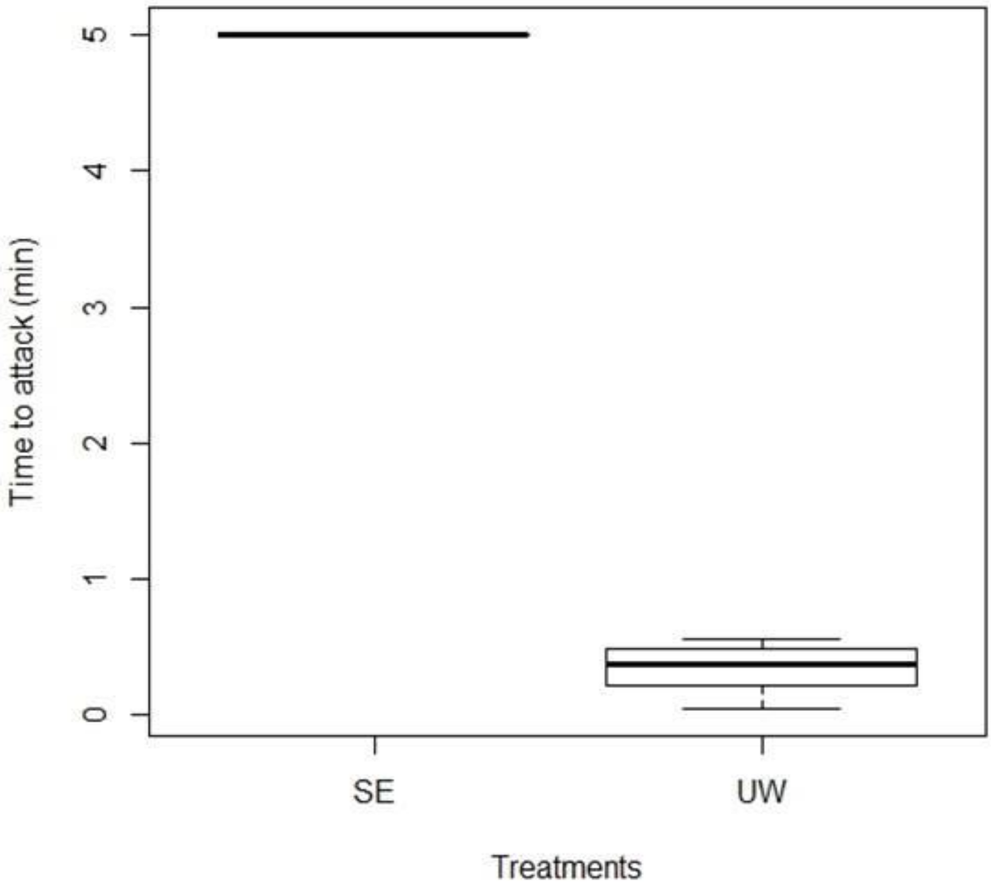

Fig. 4. Response time of the ants when exposed to individuals of *Rhinella major* coated with skin extracts (SE; N = 10) of frog and coated with ultrapure water (UW; N = 10).

CONCLUSÃO

Nós demonstramos que as formigas cortadeiras *Atta sexdens e A. laevigata* não atacam *L. lineatus*, mas atacam agressivamente outras espécies de anuros. Os indivíduos de *Rhinella major* embebidos com extratos da pele de *L. lineatus* não foram atacados quando expostos às formigas, mas indivíduos da mesma espécie embebidos com água ultrapura foram atacados. Nossos resultados suportam a hipótese que *L. lineatus* tem compostos na pele que são reconhecidos pelas saúvas *Atta* permitindo a coexistência.

Além da associação com formigas *Atta* vários aspectos sobre a ecologia e história de vida de *Lithodytes lineatus* ainda são muito escassos. Nosso estudo dá indícios de que a rã apresenta algum composto que inibi a resposta defensiva das formigas. Esse aspecto abre espaço para discussões sobre a aplicabilidade biotecnológica dos compostos presentes na secreção cutânea de *L. lineatus*, uma vez que a descrição e caracterização química e estrutural das substâncias ainda não foram realizadas.

De modo similar, o tipo de relação ecológica entre *L. lineatus* e as formigas *Atta* ainda não é bem entendido. São claras as vantagens para a rã nessa associação, mas não são conhecidos benefícios aparentes para as formigas em tê-la como parceira de ninho. Esse fato nos leva a suspeitar que se trate de uma relação comensal, porém, não se sabe se esses indivíduos se alimentam das formigas residentes, o que o tornaria um parasita nos ninhos.

Sugerimos aqui algumas possíveis explicações para as formigas tolerarem a presença da rã, caso seja uma relação mutualística. Ninhos de formigas geralmente são utilizados por outros invertebrados, dessa forma, e como também sugerido por Schluter e Regos (1981), *L. lineatus* poderia estar se alimentando destes indivíduos controlando o número de coabitantes do ninho. Outra possível explicação seria que *L. lineatus* estaria se alimentando dos inimigos naturais de formigas saúvas, como cupins, por exemplo, reduzindo a competição entre estes organismos.

APÊNDICE

PG-ECO-INPA INPA MCTI Ministério da Ciência, Tecnologia e Inovação GOVERNO FEDERAL BRASIL PÁTRIA EDUCADORA

PROGRAMA DE POS-GRADUAÇAO EM ECOLOGIA – PPG ECO

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Aos 05 dias do mês de Abril do ano de 2016, às 09h00min, no Auditório dos PPG's ATU/CFT/ECO – Campus III – INPA/V8. Reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Igor Luis Kaefer,** da Universidade Federal do Amazonas - UFAM, o(a) Prof(a). Dr(a). **Elizabeth Franklin Chilson,** do Instituto Nacional de Pesquisas da Amazônia - INPA e o(a) Prof(a). Dr(a). **Túlio de Orleans Gadelha Costa,** da Universidade Federal do Amazonas - UFAM, tendo como suplentes o(a) Prof(a). Dr(a). Renato Cintra Soares, do Instituto Nacional de Pesquisas da Amazônia - INPA, e o(a) Prof(a). Dr(a). William Ernest Magnusson, do Instituto Nacional de Pesquisas da Amazônia - INPA, sob a presidência do(a) primeiro(a), a fim de proceder a argüição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **ANDRÉ DE LIMA BARROS,** intitulado: **"QUAL O MECANISMO UTILIZADO PELA RÃ LITHODYTES LINEATUS (ANURA: LEPTODACTYLIDADE) PARA COEXISTIR COM FORMIGAS SAÚVAS DO GÊNERO ATTA (HYMENOPTERA: FORMICIDAE)?",** orientado pelo(a) Prof(a). Dr(a). Albertina Pimentel Lima do Instituto Nacional de Pesquisas da Amazônia - INPA e coorientado pelo(a). Prof(a). Dr (a). Jorge Luís López-Lozano da Fundação Alfredo da Mata – FMT. Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

- [X] APROVADO(A)
- [ ] REPROVADO(A)
- [X] POR UNANIMIDADE
- [ ] POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a).Dr(a). Igor Luis Kaefer ____________________

Prof(a).Dr(a). Elizabeth Franklin Chilson ____________________

Prof(a).Dr(a). Túlio de Orleans Gadelha Costa ____________________

Prof(a).Dr(a). Renato Cintra Soares ____________________

Prof(a).Dr(a). William Ernest Magnusson ____________________

Dra. Albertina Pimentel Lima
Coordenadora do Programa de Pós-Graduação em Ecologia – PPG ECO

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