Diversität und Ernährung von Anuren im Ökosystem Araukarienwald: Fallstudie Pró-Mata

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1. Vorspann

...und über brasilianische Froschkonzerte:

"Os caçotes são os mais líricos de todos os sapos. Reunem-se em grupos de duzentos e mais, e se distribuem em pequenas orquestras, compostas de trinta músicos cada uma. Começam o concêrto, tirando cada grupo uma nota, desde o "dó" até o "si".

Dirige o trabalho um sapo mais velho que os outros, o sapo maestro. Se, em meio do concêrto, algum sapo erra a nota, o sapo maestro faz um "dó" grave, que é o sinal para que a orquestra pare.

Parada a orquestra, ficam todos em suspenso durante alguns minutos. Depois, o concêrto continua."

"Die "caçotes"[1] *sind die lyrischsten von allen Kröten. Sie versammeln sich in Gruppen von zweihundert oder mehr und verteilen sich auf kleine Orchester, die jeweils aus dreißig Musikern bestehen. Sie beginnen das Konzert, indem jede Gruppe eine Note anklingen lässt, vom "C" bis zum "H".*

Das Konzert leitet eine Kröte, die Meisterkröte, die älter als die anderen ist. Wenn mitten im Konzert eine Kröte den falschen Ton trifft, lässt der Krötenmeister ein tiefes "C" erklingen, das Zeichen, damit das Orchester inne hält.

Einmal gestoppt, verbleiben alle für ein paar Minuten regungslos. Danach spielt das Orchester weiter."

(zit. aus SANTOS 1955)

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[1] Als "caçotes" werden in Nordbrasilien alle kleinen Frösche bezeichnet.

2. Danksagung

Für die Vergabe des Themas und die Betreuung der Arbeit danke ich Prof. Dr. Wolf Engels.

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3. Einleitung

Die Mata Atlântica Brasiliens gehört zu den „hot spots“ der Biodiversität (MYERS et al., 2000). Fauna und Flora dieses von der UNESCO als besonders schützenswert anerkannten Bioms enthalten hohe Anteile an Endemiten. In den letzten Jahrzehnten wurde dieser Küstenregenwald durch Brandrodung und Abholzung in manchen Regionen Brasiliens bis auf wenige Prozent seiner ursprünglichen Fläche reduziert (DEAN, 1995; SAATSCHI et al., 2001). Inzwischen sind viele der Restwälder unter Schutz gestellt worden. Ob die Artenvielfalt in diesen oft anthropogen schon stark beeinflussten "Inseln" erhalten werden kann, ist noch ungewiss.

Der südbrasilianische Araukarienwald stellt die montane Ausprägung der Mata Atlântica dar (KLEIN, 1960). Da das Holz der Araukarie (*Araucaria angustifolia)* sehr begehrt ist, wurden diese Nadelbäume bis in die 80er Jahre des 20. Jahrhunderts fast überall gefällt. Größere zusammenhängende Araukarienwälder existieren heute nicht mehr. Die südlichsten Wälder dieser Baumart befinden sich im Bundesstaat Rio Grande do Sul, sie gelten bis heute als kaum erforscht. Hier wurde daher mit Unterstützung durch die Universität Tübingen 1993 von der PUCRS das Centro de Pesquisas e Conservação da Natureza Pró-Mata eingerichtet und eine Waldstation erbaut, die 1996 eingeweiht werden konnte. In diesem über 5.000 h großen Areal wird in einem Langzeitprojekt das Ökosystem Araukarienwald analysiert. Es geht einerseits um Grundlagenkenntnisse für eine Wiederbewaldung und nachhaltige Forstwirtschaft, andrerseits um die Erfassung der Biodiversität sowie funktionelle Studien und allgemein um interorganismische Beziehungen (ENGELS, 2002). Die beiden letzten

Aspekte sind als Basiswissen für ein Verständnis der Evolution dieses einmaligen Tropenwald-Ökosystems mit einer Konifere als Leitart anzusehen.

Bei den zoologischen Teilprojekten stehen vor allem die Erfassung der Zusammensetzung und die ökologische Rolle solcher Tiergruppen im Vordergrund, deren Impakt für das Ökosystem als bedeutend eingestuft wird. Diese Untersuchungen hatten bisher Ameisen (DEHMER, 1997; KETTERL 2003), Bienen (CAPPELLARI, 2003; HARTER, 1999; KÖHLER, 2001), Käfer (MECKE, 2001), Raubwanzen (WEIRAUCH, 1998), Reptilien (DI-BERNARDO, 1998) und auch Amphibien (KWET, 2000, SOLÉ, 2000) zum Gegenstand. Die Ergebnisse sind im virtuellen Araukarien-Journal unter www.pro-araucaria-online.com im Internet abrufbar (ENGELS & HEINLE, 2003).

Amphibien spielen in vielen Regenwald-Ökosystemen der Neotropis als Prädatoren eine zentrale Rolle (CALDWELL, 1996; LIMA, 1998). Faktoren, die in subtropischem und gemäßigtem Klima oft die Ausbreitung von Amphibien limitieren, wie z. B. das Vorhandensein von Gewässern, sind hier zumeist von untergeordneter Bedeutung. Dagegen gibt es zahlreiche spezifische Anpassungen besonders in der Reproduktion. Einige Amphibien sind z. B. Direktentwickler geworden, aus ihren am Boden abgelegten Eiern schlüpfen fertig metamorphosierte Frösche (ROGOWITZ et al., 2001). Sie sind deshalb in der Lage, nicht nur die Nähe von Gewässern zu besiedeln, sondern kommen auch in geschlossenen Waldhabitaten vor.

Noch immer ist die Bestandsaufnahme der neotropischen Herpetofauna und insbesondere der Araukarienwälder keineswegs abgeschlossen. Die

Situation ist mit dem entsprechenden Kenntnisstand über die Amphibien Europas nicht zu vergleichen. Hier sind praktisch alle Amphibienarten seit langem bekannt (GASC et al., 1997). Im krassen Gegensatz dazu waren die südbrasilianischen Amphibien bis vor kurzem kaum untersucht. So ist nach 1980 die Zahl der für den Bundesstaat Rio Grande do Sul beschriebenen Arten von 65 (BRAUN & BRAUN, 1980) auf 76 (KWET, 2004) angestiegen, wobei zahlreiche Revalidierungen und auch Synonymisierungen notwendig wurden. Dies ist vor allem Axel Kwet zu verdanken, der eine gründliche systematisch-taxonomische Erfassung der Araukarienwald-Anuren initiierte (KWET, 2000). Die rasche Benennung noch unbeschriebener Taxa, wie sie bei Arbeiten in den Tropen über spezielle Fragestellungen nicht selten entdeckt werden, ist allein deswegen erforderlich, weil sie sonst in Publikationen überhaupt nicht angeführt werden dürfen, da sie später kaum wiederzufinden wären. Neben vielen Adultformen sind vor allem die Larven der meisten Froscharten noch gänzlich unbekannt, denn Kaulquappen sind früher kaum gesammelt worden. Bis heute steht deren Beschreibung daher weitgehend aus.

Im Rahmen meiner Promotion habe ich bei Froschlurchen über zwei Fragenkomplexe gearbeitet: Diversität und Ernährung. Beide gehören zum Tübinger Langzeit-Programm der Erforschung des Ökosystems Araukarienwald.

1. Hauptziel war die Entwicklung einer schonenden Methode zur Analyse von Mageninhalten bei Anuren, als Grundlage für Studien zu ihrer Ernährungsbiologie, mit exemplarischen Untersuchungen auf Pró-Mata.

2. Dazu die Weiterführung der systematisch-taxonomischen Erfassung der hochdiversen Fauna an Fröschen und Kröten im Araukarienwald, und diese erweitert auf die Larven.

Um die Nahrungsspektren einzelner Amphibien-Arten und deren saisonale Veränderungen sowie auch ontogenetische Diätmuster zu analysieren, sind repräsentative Stichproben erforderlich. Beobachtungen zum Jagdverhalten allein reichen hierfür keineswegs aus. Bislang wurden in ähnlichen Studien daher große Individuenzahlen von Fröschen und Kröten gesammelt, alle Tiere abgetötet und nach Sektion ihr Mageninhalt untersucht (TOFT, 1981; EVANS & LAMPO, 1996). Aus Naturschutzgründen und wegen des weltweiten Rückgangs von Amphibien-Populationen (WAKE, 1991) ist diese Vorgehensweise heute nicht mehr akzeptabel. Als Alternative bieten sich Magenspülungen an. In den wenigen mit dieser Technik bisher an Amphibien durchgeführten Untersuchungen traten aber viele Nebenwirkungen auf, die teils auch zu beträchtlichen Ausfällen führten (JOLY, 1997).

Erste Schritte dienten daher der Optimierung der „flushing“-Technik, so dass damit Analysen an einem größeren Material ohne Tierverluste möglich würden. Zahlreiche Details vom Fang der Anuren bis zur Konservierung der erhaltenen Beutereste und dem Zurückbringen der Versuchstiere wurden in vielen Varianten erprobt und verbessert, so dass nunmehr Studien zum Beutespektrum ohne Untersuchungs-bedingte Mortalität der Amphibien durchgeführt werden können. Außerdem sollten schonende Techniken für kleine Individuen entwickelt werden, denn über das Beutespektrum frisch metamorphosierter Frösche war so gut wie nichts bekannt.

Es gelang, das Verfahren insgesamt so zu optimieren, daß bei über 500 untersuchten Kröten und Fröschen praktisch keine Schädigungen auftraten. Diese Methoden wurden sogleich zur Klärung spezieller Fragen eingesetzt (Veröffentlichungen 1, 2, 3 und 11). Auch bei einer Schlangenart konnten Daten über ihre Ernährung gewonnen werden (Veröffentlichung 10).

Daneben setzte ich die systematisch-taxonomische Erfassung der Araukarienwald-Anuren fort (Veröffentlichungen 5 und 8). Hierfür wurde auch eine photographische Registrierung von Individuen bestimmter Populationen erprobt, die zumindest bei auffällig gezeichneten Arten die übliche Zehenamputation überflüssig macht und ein Wiedererkennen erlaubt. Ein interessantes Verteidigungs-Verhalten konnte bei einer bisher kaum bekannten Froschart festgestellt werden (Veröffentlichung 10).

Um überhaupt Untersuchungen zur Entwicklung und Lebensweise der Larven durchführen zu können, müssen die Kaulquappen der Araukarienwald-Anuren erst beschrieben werden. Für mehrere Frosch-Spezies konnten kurze morphologische Erstbeschreibungen ihrer Larven vorgenommen werden, verbunden mit Freiland-Beobachtungen (Veröffentlichungen 5, 6 und 7).

Bei meinen Studien zur Ernährungsbiologie der im Araukarienwald und angrenzenden Habitaten vorkommenden Anuren bin ich von der Hypothese ausgegangen, dass bei dieser hochdiversen Artengemeinschaft der interspezifische Konkurrenzdruck durch Spezialisierung auf bestimmte Beutespektren verringert sein sollte. Dies galt es quantitativ zu erfassen. Zu fragen war:

Wenn eine Spezialisierung vorliegt, betrifft sie die Jagdweise (sit- and wait-Prädatoren *versus* aktive Jäger) oder handelt es sich um eine selektive Aufnahme bestimmter Nahrungselemente?

Nehmen die Froschlurche über das ganze Jahr hinweg Nahrung zu sich oder unterbrechen sie die Jagd während ihrer Fortpflanzungsperiode?

4. Material und Methoden

Die verwendeten Materialien und Untersuchungsmethoden sowie eine genaue Beschreibung des Untersuchungsgebietes Pró-Mata sind in den einzelnen Publikationen zu finden.

5. Ergebnisse

Die Ergebnisse meiner Untersuchungen sind in folgenden Publikationen enthalten:

5.1 **Solé, Mirco**; Ketterl, Jochen; Di-Bernardo, Marcos & Kwet, Axel (2002): Ants and termites are the diet of the microhylid frog *Elachistocleis ovalis* (Schneider, 1799) at an Araucaria forest in Rio Grande do Sul, Brazil. Herpetological Bulletin 79, 14 – 17

5.2 **Solé**, **Mirco**; Beckmann, Olaf; Pelz, Birgit; Engels, Wolf & Kwet, Axel: Stomach-flushing for Diet Analysis in Anurans: an Improved Protocol Evaluated in a Case Study in *Araucaria* Forests, Southern Brazil. Studies on Neotropical Fauna and Environment (2004, im Druck).

5.3 **Solé, Mirco**; Pelz, Birgit; Di-Bernardo, Marcos & Kwet, Axel: Do male tree frogs feed during the breeding season? A comparison of five hylid species in Rio Grande do Sul, southern Brazil. Revista Española de Herpetologia (2004, eingereicht).

5.4 Kwet, Axel & **Solé**, **Mirco**: Taxonomic status of *Hylodes henselii* Peters, 1870 from southern Brazil, and description of acoustic variation in *Eleutherodactylus guentheri* (Anura: Leptodactylidae). Journal of Herpetology (2004, im Druck).

5.5 Both, Camila; Kwet, Axel & **Solé, Mirco**: The tadpole of *Physalaemus lisei* Braun & Braun, 1977 from southern Brazil (Anura: Leptodactylidae). Alytes (2004, eingereicht).

5.6 Both, Camila; Kwet, Axel & **Solé, Mirco**: The tadpole of *Hyla leptolineata* (Braun & Braun 1977), a species in the *Hyla polytaenia* group (Anura, Hylidae). Brazilian Journal of Biology (2004, eingereicht).

5.7 Carvalho, Geraldo, **Solé, Mirco** & Kwet, Axel: Descrição do girino de *Leptodactylus plaumanni* AHL, 1936 (Anura, Leptodactylidae) do Centro de Pesquisa e Conservação da Natureza Pró-Mata, Rio Grande do Sul, Brasil. Caderno de Pesquisa série Biologia (2004, eingereicht).

5.8 Miranda, Tatiana; Ebner, Martin; **Solé, Mirco** & Kwet, Axel: Estimativa populacional de *Pseudis cardosoi* (Anura, Hylidae), com emprego de Método Fotográfico para reconhecimento individual. Biociências (2004, im Druck).

5.9 **Solé**, **Mirco** & Kwet, Axel (2003): *Liophis jaegeri* (Jaeger´s Ground Snake) Diet. Herpetological Review 34: 69.

5.10 Kwet, Axel & **Solé**, **Mirco** (2002): *Elachistocleis erythrogaster* (Redbellied Oval Frog). Defensive Behavior. Herpetological Review 33: 46.

5.11 **Solé**, **Mirco**; Pelz, Birgit & Kwet, Axel (2004): *Hyla faber* (Smith frog) Diet. Herpetological Review 35: 159.

Bei den 5 Hauptarbeiten zum Thema Ernährung bin ich Erstautor.

6. Diskussion

In den erstmals mittels Magenspülungen vorgenommenen Analysen der Nahrungsgrundlagen von auf dem Araukarienplateau von Rio Grande do Sul lebenden Anuren wurde unser optimiertes Verfahren erfolgreich eingesetzt, womit ein Abtöten zahlreicher Tiere überflüssig wurde.

Die Ergebnisse bestätigen meine Hypothese einer weitgehenden Spezialisierung im Beutespektrum und damit in der Ernährung, was für mehrere der häufigen Kröten- und Froscharten eindeutig belegt werden konnte. Für verschiedene Froscharten sind Ameisen die hauptsächliche Nahrungsgrundlage. Dies ist auch für viele Frösche aus dem Amazonasgebiet bekannt (VITT & CALDWELL, 1994; CALDWELL, 1996). Für den Engmaulfrosch *Elachistocleis bicolor* gilt sogar, daß Ameisen und Termiten die ausschließliche Beute darstellen. Allerdings werden keineswegs wahllos sämtliche Ameisen (es gibt auf Pró-Mata weit über Hundert Arten!) gefressen, sondern nur solche, die nicht mit Ameisensäure spritzen bzw. danach riechen. Das bedeutet, dass Formicinen als Beute verschmäht werden. Bei bestimmten Froscharten löst Ameisensäure sogar eine spezifische Abwehr-Reaktion aus (KOKUBUM & MENIN, 2002).

Die meisten Pfeiffrösche erbeuten allgemein bodenbewohnende Arthropoden, ihr nicht sehr enges Nahrungsspektrum umfasst ebenfalls Ameisen, daneben aber auch viele kleinere Käfer. Aufgrund der überaus reichen epigäischen Mesofauna scheinen hier keine scharfe Konkurrenz-Situation und folglich auch kein großer Spezialisierungsgrad vorzuliegen. Diese Ergebnisse stimmen mit den Befunden für amazonische Blattstreubewohner (LIMA & MAGNUSSON, 2000), Pfeiffrösche aus dem

Atlantische Küstenregewald (VAN SLUYS et al., 2001) und Anuren der Wälder Venezuelas (DUELLMAN, 1997), überein.
Bei den Laubfröschen unterscheiden sich die vorwiegend festgestellten Nahrungselemente bei arborealen und terrestrischen Spezies. Die Baumbewohner fressen vor allem Käfer, Spinnen und Zikaden, während die Frösche der Grasland-Arten, wie z. B. *Hyla pulchella*, neben Käfern auch viele Schmetterlinge und Hautflügler erbeuten. Dies interpretiere ich als Jagdhabitat-spezifische Einnischung in der Ernährungsweise. Die kongenerischen Spezies haben bei übereinstimmender aktiver Jagdstrategie durch Anpassung an unterschiedliche Strata als Lebensraum offensichtlich eine Konkurrenz-Vermeidung auf räumlicher Ebene erreicht. Die Evolution hat also eine spezifische Habitat-Bindung ergeben.

Soweit die Freilandbeobachtungen und die Auswertung der Mageninhalte dies zulassen, können einige der untersuchten Anuren-Arten als „sit-and-wait" Prädatoren eingestuft werden. Sie erbeuten opportunistisch Tiere passender Größe, die in ihre Nähe geraten. Ausnahmen hiervon sind vor allem die myrmekophagen Nahrungsspezialisten, die ihre Beute selektieren.

Wie sieht es aber hinsichtlich saisonaler Unterschiede in der Ernährung aus? Auf Pró-Mata kann es im Winter, also im Juli und August, recht kalt sein, mit nächtlichen Frösten und gelegentlichem Schneefall. Natürlich ruhen alle Amphibien während dieser Zeit, eine Nahrungsaufnahme entfällt. Dagegen gibt es während der warmen Jahreszeiten durchaus klare Abhängigkeiten der Jagdaktivität und des Fressverhaltens vom Reproduktions-Zyklus, wenn auch keineswegs bei allen Arten. Hier spielt die spezifische Länge der Fortpflanzungsperiode eine große Rolle. Ist sie kurz, dann nehmen die Frösche, vor allem die rufenden Männchen, in dieser Zeit meist keine Nahrung zu sich. Ist sie dagegen ausgedehnt, dann wird durchaus auch gejagt, und die Mägen enthalten regelmäßig das arttypische Spektrum an Nahrungselementen.

Nach den bisherigen Befunden sind in erster Linie Arthropoden also die Nahrungsgrundlage vieler Kröten und Frösche des Araukarienwaldes. Trotz Durchführung der Magenspülungen kurz nach dem Fang stellt die bei Amphibien sofort und intensiv einsetzende Verdauung der Mageninhalte ein Problem für die Identifizierung aller Nahrungskomponenten dar. Vor allem von adulten Insekten sind chitinhaltige Reste der Cuticula, Elytren bei Käfern, Mandibeln und andere Hartteile gut zu bestimmen. Schwierigkeiten bereiten die Reste von Insektenlarven wie Raupen, sowie von Beuteobjekten wie Regenwürmern oder Nacktschnecken. Wahrscheinlich sind sie daher in der Liste der Beutespektren unterrepräsentiert. Dies hat bei einigen Anuren vielleicht zu einem unvollständigen Bild geführt. Eventuell könnte bei künftigen Untersuchungen probiert werden, ob mit molekularen Sonden eine Komplettierung der Diätlisten erreicht werden kann.

Ausgewachsene Individuen der großen Anuren-Arten fressen durchaus auch andere Amphibien, zumindest gelegentlich. Dies gilt beispielsweise für den sog. Schmied (*Hyla faber*). Diese Art ist einer der größten Laubfrösche der Neotropis überhaupt. Die Adulten fressen kleinere Laubfrösche, wobei dieser Teil ihres Beutespektrums mehrere Spezies umfasst, wohl ab und zu auch kleinere Artgenossen. Auch für den Pfeiffrosch *Leptodactylus ocellatus* konnte die Vermutung von MANEYRO et al. (2004), dass es sich um eine anurophage Art handeln könnte, bestätigt werden.

Für wen sind darüber hinaus Anuren reguläre Beute? Daß Schlangen Frösche und Kaulquappen jagen, ist bekannt und war auch für das an Reptilien reiche Untersuchungsgebiet anzunehmen (DI-BERNARDO, 1998). Dass einige Schlangen Froscheier fressen war auch bekannt (VITT, 1996). Nicht bekannt war bisher, dass Schlangen auch Schaumnester von Fröschen fressen. Nach entsprechenden zufälligen Freilandbeobachtungen wurde hierzu ein Laborversuch angesetzt. Eine Buntnatter der Art *Liophis jaegeri* fraß dabei ohne weiteres ein Schaumnest des Pfeiffrosches *Leptodactylus plaumanni*. Dies legt nahe, dass auch die Nester anderer Vertreter der Familie Leptodactylidae von Buntnattern gefressen werden können. Weil dabei wohl oft ein komplettes Gelege verloren geht, dürfte dieses hier erstmalig beschriebene Verhalten durchaus von Bedeutung für den Räuberdruck auf Pfeiffrösche eingestuft werden.

Die meist kurzen Veröffentlichungen zum taxonomischen Status einer *Eleutherodactylus*-Art sowie über die Kaulquappen mehrerer Froschspezies sind als Beiträge zur Erfassung der herpetologischen Biodiversität auf dem Araukarien-Plateau einzuordnen. Sie entstanden am Rande der analytischen und experimentellen Untersuchungen. Teils handelt es sich um dabei um von mir betreute Teilprojekte brasilianischer Mestrado-Kandidaten. Es wurde u. a. geprüft, ob für Kaulquappen die Zahnformel als Bestimmungsmerkmal dienen kann, was sich jedoch als nicht immer unterscheidbar herausstellte. Um hier zu einer abschließenden Bewertung zu kommen, müssen allerdings noch weit mehr Anuren-Larven untersucht werden.

Wenngleich es sich bei diesem Bereich meiner Dissertation um eine ganze Reihe von Titeln handelt, betrachte ich doch die Veröffentlichungen zur Ernährungsbiologie als den eigentlichen Schwerpunkt meiner Doktorarbeit. Der Aufwand hierfür war beträchtlich, und meine Eigenleistung in diesen Untersuchungen überwiegt bei allen hierzu rechnenden Publikationen.

Diese Befunde bewerte ich als notwendigen Teil jedweder biologischen Arbeit in Gebieten der Erde, deren faunistische Inventur als noch nicht abgeschlossen anzusehen ist. Reine Biodiversitäts-Studien mit dem Ziel, Artenlisten zu erstellen, sind nur bei allerersten Bestandsaufnahmen einer *terra incognita* gerechtfertigt, und solche Areale gibt es durchaus noch in vielen abgelegenen Gebieten oder großräumigen Waldhabitaten, einschließlich vieler Tropenwälder. Für ein Verständnis der Ökosysteme sind jedoch analytische Studien die Voraussetzung. Wenn sich beides sinnvoll verknüpfen lässt, wie ich es bei meiner Doktorarbeit realisieren konnte, macht das Ergebnis vielleicht den Eindruck einer Aneinanderreihung wissenschaftlicher Beiträge, die nicht sämtlich unter nur einer Fragestellung subsummiert werden können. Jedoch stand als Ausgangspunkt aller meiner Untersuchungen die Frage nach den interorganismisch so vielseitig verknüpften Nahrungsketten und den vernetzten Ernährungsgrundlagen der Amphibien im erst so wenig erforschten Ökosystem Araukarienwald im Vordergrund.

7. Eigenanteil

Die Eigenanteile der Veröffentlichungen meiner Dissertation betragen bei

3.1 = 80 %

Die Frösche wurden im Untersuchungsgebiet von mir gesammelt, vermessen, gewogen und der Magen ausgespült. Die dabei hauptsächlich erhaltenen Ameisen wurden in Zusammenarbeit mit Jochen Ketterl bestimmt. Konzipierung, Aufbau und Durchführung der Versuche zur Reaktion der Frösche auf bestimmte angebotene Ameisen erfolgte durch mich, ebenso wie die Auswertung der Daten und das Verfassen des Artikels. Marcos Di-Bernardo und Axel Kwet gaben dazu Ratschläge, Robert Paxton und zwei anonyme Gutachter verbesserten die sprachliche Qualität.

3.2 = 85 %

Die untersuchten Kröten (*Bufo ictericus*) wurden von Olaf Beckmann und mir, die Laubfrösche von Birgit Pelz und mir gefangen; alle Tiere wurden unter meiner Anleitung im Labor gemeinsam untersucht. Sämtliche übrigen Anuren sammelte und untersuchte ich allein. Die Optimierung der Magenspülung in vielen der bisher üblichen Einzelschritte führte ich durch, dies erforderte einen erheblichen Aufwand. Das Ergebnis ist ein schonendes, von einer Versuchsperson durchführbares Verfahren ohne erkennbare Schädigung der untersuchten Tiere, und dies bei einem guten Erhaltungszustand der Mageninhalte. Die Auswertung der gesamten Mageninhalte und der so erhaltenen Daten wurden von mir durchgeführt, beraten von Axel Kwet und Wolf Engels. Korrekturvorschläge zum Manuskript erfolgten durch Anne Zillikens als Schriftleiterin sowie zwei Gutachter.

3.3 = 70 %

Die Laubfrösche wurden von Birgit Pelz und mir gesammelt und im Labor unter meiner Anleitung untersucht. Die Bestimmung der Mageninhalte erfolgte zunächst durch Birgit Pelz, sie wurde von mir überprüft und verifiziert. Die Auswertung der Daten und die Abfassung des Manuskriptes erfolgten gemeinsam.

3.4 = 40 %

Die Rufe der Männchen wurden im Freiland von Axel Kwet aufgenommen und im Labor ausgewertet. Das Material der Sammlungen in Saarbrücken (BGSS), Wien (NMW), Stuttgart (SMNS), Washington (USNM) und Berlin (ZMB) wurde von Axel Kwet, das der Museen in Porto Alegre (MCT), La Plata (MLP), Buenos Aires (MACN, CENAI) und Rio de Janeiro (MNRJ) von mir gesichtet und vermessen. Ein weiterer Teil der Frösche wurde von mir im Waldschutzgebiet Pró-Mata gesammelt und fixiert. Das Manuskript wurde von Axel Kwet entworfen und von mir ergänzt. Ronald Heyer und Marcelo Sánchez korrigierten die erste Fassung.

3.5 = 50 %

Die Kaulquappen für die Beschreibung wurden von Axel Kwet und mir im Waldschutzgebiet Pró-Mata aus Eiern eines Paares in eigens angelegten Kleingewässern aufgezogen und Stichproben wichtiger Stadien fixiert. Die Zeichnungen der Larven und ihrer Mundpartien wurden von Camila Both angefertigt. Die Schnitte für die elektronenmikroskopischen Aufnahmen wurden von mir erstellt, die Fotos (mit technischer Hilfe) von Camila Both und mir angefertigt und gemeinsam ausgewertet. Die Endfassung des Manuskriptes wurde von Axel Kwet und Rafael de Sá beraten.

3.6 = 50 %

Die Kaulquappen wurden von Axel Kwet im Freiland gesammelt. Alle Zeichnungen fertigte Camila Both an. Die Präparate für die elektronenmikroskopischen Analysen wurden von mir angefertigt. Die Aufnahmen machten (mit technischer Assistenz) Camila Both und ich gemeinsam. Die Beobachtungen im Freiland zur Fortpflanzungsbiologie sowie das Sammeln und Auszählen der Schaumnester führte ich durch. Die Auswertung der Daten sowie das Verfassen des Manuskripts wurden von Camila Both und mir gemeinsam vorgenommen. Paula Eterovick beriet uns bei der Endfassung des Manuskriptes.

3.7 = 50 %

Die Kaulquappen wurden vom Ei ab von mir im Waldschutzgebiet Pró-Mata in eigens dafür angelegten Wasserlöchern aufgezogen, im Abstand von 5 Tagen wurden Stichproben fixiert. Die Zeichnungen wurden von Geraldo Carvalho angefertigt. Alle Freilandbeobachtungen zur Fortpflanzungsbiologie wurden von mir im Waldschutzgebiet Pró-Mata durchgeführt. Das Manuskript wurde von Geraldo Carvalho entworfen, die Endfassung wurde von Axel Kwet und mir erstellt.

3.8 = 40 %

Die Idee, Individuen des auffällig gezeichneten Harlekinfrosches *Pseudis cardosoi* an Hand von Fotos wieder zu erkennen, stammte von mir. Das Sammeln und Fotografieren der Tiere im Freiland führten Tatiana Miranda, Martin Ebner und ich gemeinsam durch. Martin Ebner entwickelte dabei wesentliche technische Vorraussetzungen des Verfahrens. Das Manuskript wurde von Tatiana Miranda entworfen, die Endfassung beruht auf Vorschlägen von Martin Ebner, Axel Kwet und mir.

3.9 = 80 %

Im Freiland beobachtete ich erstmals, daß Schaumnester von Fröschen bestimmten Schlangen als Nahrung dienten. Dieser Befund wurde experimentell im Labor bestätigt. Zu der hierüber von mir verfaßten Kurzmitteilung steuerte Axel Kwet einige Literaturhinweise und Ideen bei.

3.10 = 50 %

Die Beobachtungen an den Fröschen, die Sammlung des Materials und das Verfassen der Kurzmitteilung führten Axel Kwet und ich gemeinsam durch.

3.11 = 80 %

Die Beobachtungen und das Sammeln der Frösche im Freiland führten Birgit Pelz, Marcus Wittmann und ich gemeinsam durch. Die Magenspülungen nahmen Birgit Pelz und ich vor. Die Auswertung der Proben und das Verfassen des Manuskripts der Kurzmitteilung stammen im Wesentlich von mir, unter Beteiligung von Axel Kwet und Birgit Pelz.

8. Zusammenfassung

Insgesamt 5 Veröffentlichungen behandeln das Hauptthema, die Nahrungsgrundlagen der auf dem Araukarienplateau von Rio Grande do Sul lebenden Anuren. Hierfür wurden Magenspülungen eingesetzt, wobei dieses Verfahren in umfangreichen methodischen Untersuchungen zunächst so optimiert wurde, dass schließlich ohne Mortalität der Versuchstiere einschließlich kleiner Tiere ab ca. 20 mm Körpergröße gearbeitet werden konnte. Meine Arbeitshypothese einer Spezialisierung in der Nutzung des breiten Angebots an Beute konnte verifiziert werden. Auch begrenzte Jagdhabitate führen offensichtlich zu einer Konkurrenz-Vermeidung. Viele der untersuchten Arten können als sit-and-wait Prädatoren charakterisiert werden. Vorherrschende Beuteobjekte waren Arthropoden. Bei einzelnen größeren Spezies spielen auch andere Frösche eine Rolle im Nahrungsspektrum. Saisonale Unterschiede in der Nahrungsaufnahme sind vor allem bei Arten mit kurzer Fortpflanzungsperiode ausgeprägt. In dieser Zeit waren die Mägen durchweg leer. Einige Engmaulfrösche erwiesen sich als ausgesprochene Nahrungsspezialisten, sie fressen ausschließlich Ameisen und Termiten. Allerdings werden keineswegs Ameisen aller Arten akzeptiert. Gemieden werden solche der Unterfamilie Formicinae, die über Ameisensäure verfügen, die bei den untersuchten Microhyliden eine charakteristische Abwehrreaktion auslöst.

Erstmals wurde bei Schlangen das Fressen von Schaumnestern beobachtet, die bei einigen Froscharten zum Ablaichen angelegt werden. Welche Bedeutung dieser Räuberdruck quantitativ für die betreffenden Froscharten hat, bleibt zu klären.

Unter den Beiträgen zur Erfassung der Anuren-Diversität sind mehrere Kurzmitteilungen morphologisch-taxonomischen Beschreibungen von Kaulquappen gewidmet. Bisher fehlten Kenntnisse über die Larven fast aller Anuren des Araukarienwaldes.

Vor allem die gewonnenen Erkenntnis über die Ernährung der Araukarienwald-Anuren ergänzen unser derzeitiges Bild dieses Ökosystems mit neuen Teilaspekten. Regenwälder sind immer Froschparadiese. Der Anteil der Kröten und Frösche am Umsatz der animalischen Biomasse muß als beträchtlich eingestuft werden. Quantitativ stehen Arthropoden als Beute sicher im Vordergrund. Die Rolle sonstiger Invertebraten kann erst beurteilt werden, wenn deren Reste in den Mageninhalten besser identifiziert werden können.

Insgesamt stellen herpetologische Studien einen wesentlichen Beitrag zum Verständnis der Evolution des Ökosystems Araukarienwald dar.

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5.1 **Solé**, **Mirco[1,2]**; Ketterl, Jochen[1,2]; Di-Bernardo, Marcos[3] & Kwet, Axel[1,2,3,4]

Ants and termites are the diet of the microhylid frog *Elachistocleis ovalis* (Schneider, 1799) at an *Araucaria* forest in Rio Grande do Sul, Brazil.

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Elachistocleis ovalis ist ein Vertreter der Engmaulfrösche (Microhylidae), über dessen Lebensweise wenig bekannt war. Der unterirdisch lebende Frosch kommt nur zur Fortpflanzungszeit für wenige Tage an kleine Tümpel. An einem solchen Habitat habe ich 16 adulte Frösche gesammelt und deren Magen ausgespült. Die Nahrungsreste wurden identifiziert und es stellte sich heraus, dass *Elachistocleis ovalis* auf das Erbeuten von Termiten und Ameisen spezialisiert ist. Ein Laborversuch mit 10 Fröschen zeigte, dass bestimmte Ameisen nicht gefressen werden, beim Anbieten von Vertretern der Unterfamilie Formicinae sogar ein bisher unbeschriebenes Abwehrverhalten gezeigt wird. Bei diesem wölben die Frösche ihren Rücken zu einem Buckel und versuchen den Kopf in das Substrat zu graben. Bei der Durchführung der Fressversuche wurde die Zeit gemessen, die zwischen dem Anbieten der Nahrung und dem Zuschnappen des Frosches verging. Dabei wurde beobachtet, dass die Frösche während der ersten drei Sekunden ihre Nasenlöcher heftig bewegten und erst danach nach dem angebotenen Futterelement schnappten. Dies ist ein Hinweis darauf, dass bei der Nahrungsauswahl dieser grabenden Art olfaktorische Prüfung beteiligt ist.

ANTS AND TERMITES ARE THE DIET OF THE MICROHYLID FROG *ELACHISTOCLEIS OVALIS* (SCHNEIDER, 1799) AT AN *ARAUCARIA* FOREST IN RIO GRANDE DO SUL, BRAZIL

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ABSTRACT.— The diet of *Elachistocleis ovalis* was studied based on material from the Serra Geral in the Brazilian state of Rio Grande do Sul. Stomachs of 16 frogs captured in their natural environment were flushed. *Elachistocleis ovalis* had an insectivorous diet composed exclusively of Hymenoptera and Isoptera. Ants of the subfamilies Myrmicinae, Dolichoderinae, Ponerinae and Ecitoninae were the most common prey items. Feeding experiments in the laboratory showed that *E. ovalis* has a defensive reaction which consists of crouching and attempting to burrow its head into the ground when offered ants of the subfamily Formicinae.

THE anuran fauna of the subtropical Rio Grande do Sul in the south of Brazil is remarkably diverse. Braun & Braun (1980) recorded 62 species and subspecies for this state. The most recent checklist (Kwet, 2001) comprises 79 species. The greatest number of species in the region is found in the state's northeastern mountains of the Serra Geral, at altitudes of around 1000 m, and on the adjacent Atlantic-facing slopes. About 50 species of frogs and toads occur in the rain forest of these upland areas (Kwet, 2000). The local anuran community has been intensively studied, and 36 taxa alone have been found in the *Araucaria* forest reserve Pró-Mata (Kwet & Di-Bernardo, 1999). Most of these species belong to the Hylidae (19) and Leptodactylidae (12) with, in addition, two microhylids, two bufonids and one pseudid. The two Microhylidae are *Elachistocleis erythrogaster* Kwet & Di-Bernardo, 1998 and *E. ovalis* (Schneider, 1799). The taxonomic status of the latter is unresolved (Frost, 1985) and we follow Klappenbach & Langone (1992) and Kwet & Di-Bernardo (1998, 1999) in considering *E. bicolor* (Valenciennes, 1838) a junior synonym of *E. ovalis*. Data available on the natural history of this small microhylid are limited and little is known about its diet. Cei (1980) called the species termitophagous whereas Langone (1994) mentioned ants as typical prey. Kwet & Di-Bernardo (1999) described *E. ovalis* as a fossorial form, feeding mainly on termites and ants. In order to obtain more precise information on presumed prey selection, we studied the stomach contents of this frog at the Pró-Mata reserve.

MATERIALS AND METHODS

The study was carried out December 2000 through March 2001 in the Pró-Mata reserve, located in the municipality of São Francisco de Paula, Rio Grande do Sul, at 29°S and 50°W. At the collecting site near the entrance of the reserve are small temporary ponds in an area of open grassland (campos); these fill with water after rains in springtime and may dry out in summer. Average annual precipitation, as measured at the nearby reserve's weather station, is about 2200 mm.

Sixteen adult *E. ovalis* were collected after heavy rainfall. Their stomachs were flushed as described by Patto (1998) and prey items identified under a stereomicroscope to genus level. In a feeding experiment, ten frogs were kept for 48 hrs in a terrarium at the field station. All specimens were placed in separate plastic boxes measuring 10 x 10 x 8 cm, containing earth and small stones as hiding places. After a period of two hours, all frogs had burrowed into the earth with only their snouts visible. Prey tests were conducted with ants of the nine most abundant species at Pró-Mata (four Myrmicinae, two Formicinae, one Ecitoninae, one Ponerinae and one Dolichoderinae) and termites of two species. Single prey items were offered with forceps to the frogs at 5 mm distance from the head and left in this position for ten seconds. If the prey was not taken during this time, it was removed and the next item was presented. The tests were run at intervals of one minute. If a frog attempted to feed upon the item, its reaction time was recorded.

RESULTS

In most of the frogs (15 out of 16), the flushed stomachs contained food. On average about 10 prey items were found per stomach. The maximum content comprised remnants of 34 insects. One stomach contained only one prey item. Based on the identification of 151 items, 10 prey categories could be distinguished (Table 1). Ants

Prey taxa	***n***	**%**
Insecta		
Hymenoptera		
Dolichoderina		
Linepithema	12	8
Ecitoninae		
Neivamyrmex	14	9
Formicinae		
Paratrechina	4	3
Myrmicinae		
Cyphomyrmex	25	16
Pheidole	22	15
Solenopsis	20	13
Wasmannia	1	1
Ponerinae		
Gnamptogenys	1	1
Hypoponera	2	1
Isoptera	50	33
Total	**151**	**100**

Table 1. Stomach prey content of 16 adult *Elachistocleis ovalis*. The four most frequent (>10%) items are given in bold italics.

Feeding tests with individual frogs	**Prey items presented to the frogs, adult *Elachistocleis ovalis*, and the lag time in seconds until the insect was captured**										
	Solenopsis saevissima	***Pheidole* sp.**	***Acromyrmex crassispinus***	***Cyphomyrmex* sp.**	***Camponotus* sp.**	***Brachymyrmex* sp.**	***Neivamyrmex* sp.**	***Hypoponera* sp.**	***Linepithema humile***	**Termite sp.1**	**Termite sp.2**
1	**6**	**7**	-	-	**dr**	**dr**	-	**6**	-	-	-
2	**7**	**8**	-	-	**dr**	**dr**	**3**	**5**	-	-	**3**
3	**5**	**6**	-	-	**dr**	**dr**	-	**6**	-	-	**3**
4	**5**	**9**	-	-	**dr**	-	-	-	-	-	**6**
5	**10**	-	-	-	**dr**	**dr**	**10**	**10**	-	-	**8**
6	**3**	**5**	-	-	**dr**	**dr**	**4**	**4**	-	-	**7**
7	**6**	**6**	-	-	**dr**	**dr**	-	**8**	-	-	**8**
8	-	**5**	-	-	**dr**	**dr**	-	**10**	-	-	**6**
9	**5**	**6**	-	-	**dr**	**dr**	-	**8**	-	-	**6**
10	-	**3**	-	-	**dr**	**dr**	**9**	-	-	-	**3**

Table 2. Reactions of 10 laboratory-maintained adult *Elachistocleis ovalis* to live ants and termites. The test items were offered in sequence to the same frog, and thereafter the next frog was tested. For those items not accepted by the frogs, their alternative reaction, as dr = defensive reaction or - = no feeding attempt, was recorded.

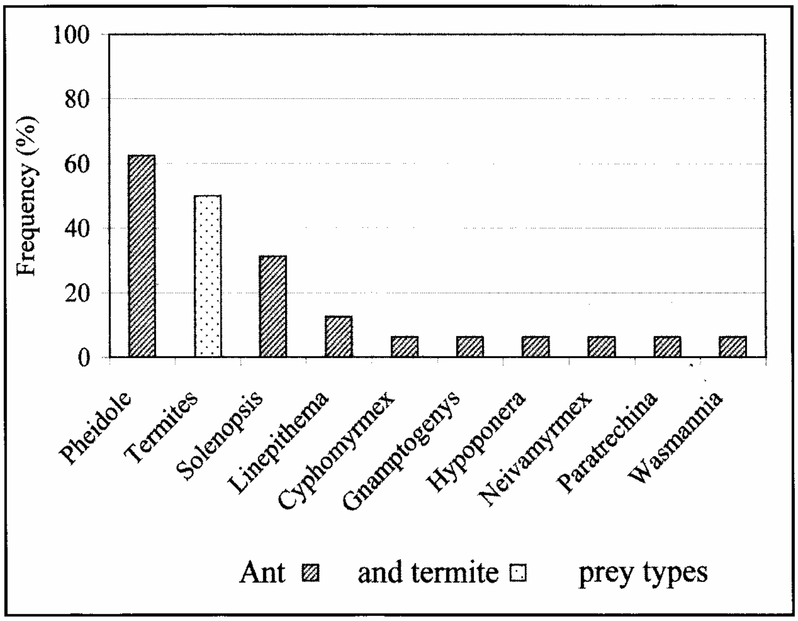

Figure 1. Diet composition in *Elachistocelis ovalis*.

predominated in the diet, representing (2/3) of all items. All the rest were termites. The latter were found in half of the stomachs. Of the ants, workers of two taxa were frequently found: about (2/3) of the stomachs contained *Pheidole* and about (1/3) *Solenopsis* ants (Figure 1).

In our feeding experiments, most of the ten adult *E. ovalis* accepted ants of the subfamilies Myrmicinae, Ponerinae and Ecitoninae. In contrast, when ants of the subfamily Formicinae were presented, they showed defensive behaviour, which consisted of crouching and attempting to burrow their head into the earth. Of the two undetermined species of termite, the workers of one were accepted whereas the others were ignored. The minimum time from presenting the prey until the frog made a feeding attempt was three seconds. During the first three seconds fast movement of the nostrils were observed in all frogs, suggesting that prey selection in *E. ovalis* is in part olfactorial.

DISCUSSION

At our study site, the south Brazilian *Araucaria* forest reserve Pró-Mata, the diet of adult *Elachistocleis ovalis* was found to consist of ants and termites only. Ants of the genera *Pheidole* and *Solenopsis* were the most frequent prey. Only two stomachs contained ants of the genus *Cyphomyrmex*; one individual contained 23 *Cyphomyrmex*. Two species of this genus, *minutus* and *olitor*, are common in Pro-Mata and occur syntopically. In one stomach, remnants of 14 individuals of the ant genus *Neivamyrmex* were found, a group of relatively rare taxa due to their nomadic life (Gotwald, 1995).

According to our data based on stomach contents, the microhylid *Elachistocleis ovalis* should be regarded an ant and termite specialist, as indicated by Simon & Toft (1991). Specialisation on ant prey is documented for various species of the subfamily Microhylinae, e.g., *Chiasmocleis ventrimaculata*, *Hamptophryne boliviana* (Schlüter & Salas, 1991) and *Microhyla ornata* (Hirai & Matsui, 2000), and may be a common pattern of nutrition in adults of this group.

In our feeding experiments, *Elachistocleis ovalis* accepted ants of the subfamilies Myrmicinae, Ponerinae and Ecitoninae. However, they showed defensive reactions towards Formicinae which may be due to the strong formic acid secretion in ants of this subfamily. Similar defensive behaviour when handled has been observed in *E. erythrogaster* (Kwet & Solé, in press). Defensive behaviour against ants has been also recorded for the African microhylid *Phrynomantis microps* (Rödel & Braun 1999) when placed in containers with the ant species *Paltothyreus tarsatus*; the frogs immediately crouched and, after some seconds, elevated the posterior part of the body and laid their heads between their forelegs.

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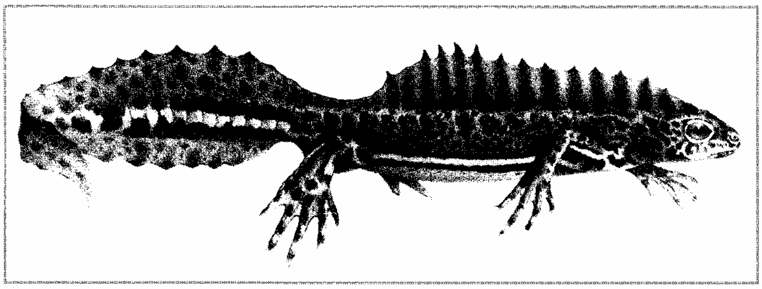

Molga vittata (= *Triturus vittatus*). From *Proceedings of the Zoological Society of London*, 1896. Reproduction courtesy of the Zoological Society of London

5.2 **Solé**, **Mirco**[**1,2**]; Beckmann[1,2], Olaf; Pelz, Birgit[1,2]; Kwet, Axel[1,2,3] & Engels, Wolf[1,2]

Stomach-Flushing for Diet Analysis in Anurans: an Improved Protocol Evaluated in a Case Study in *Araucaria* Forests, Southern Brazil

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Untersuchungen über die Ernährung von Fröschen wurden bislang mittels Aufpräparieren der Mägen von konservierten Tieren durchgeführt. Obwohl schon 1979 eine bei Schildkröten ausprobierte Magenspülungsmethode auch für Amphibien beschrieben wurde, werden bis heute für Magenuntersuchungen die Tiere in der Regel getötet. Wir haben im Laufe von 5 Jahren die Methode der Magenspülung weiterentwickelt und an 583 Fröschen von 15 Arten getestet. Die Überlebensrate lag über 99 %. Wir beschreiben anhand von Fotos eine Arbeitsweise, die von einem einzelnen Forscher durchgeführt werden kann. Die von uns weiterentwickelte Methode kommt ohne Narkose aus. Zum Spülen werden weiche Schläuche und Teichwasser benutzt Die wichtigsten Nahrungselemente werden für die untersuchten Arten angegeben. Während sich in den Mägen der meisten Pfeiffrösche Ameisen und Käfer befanden, enthielten die Mägen der Laubfrösche viele Zikaden, Spinnen und Hautflügler. Aus den Mägen der einzigen untersuchten Krötenart konnten vor allem Käfer, Ameisen und Tausendfüssler gespült werden. Die rein aquatisch lebende Harlekinfroschart *Pseudis cardosoi* ernährt sich vor allem von Käfern, Libellenlarven und Hautflüglern. Um zu untersuchen ob während der Magenspülung lebensbedrohliche innere Verletzungen entstehen, wurden 29 Frösche nach erfolgter Magenspülung einen Monat lang im Labor gehalten. Alle Frösche überlebten und nahmen regelmäßig die angebotene Nahrung auf. In Anbetracht des weltweiten Rückgangs der Amphibien empfehlen wir, die von uns weiterentwickelte Methode als Standard bei Magenuntersuchungen einzusetzen, um keine Tiere abtöten zu müssen.

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ORIGINAL ARTICLE

Stomach-flushing for diet analysis in Anurans: an improved protocol evaluated in a case study in *Araucaria* Forests, Southern Brazil*

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Abstract
Stomach-flushing is a gentle treatment in analysing the diet of vertebrates. We applied this method in the study of seasonal nutrition of anurans of 15 species at an *Araucaria* forest habitat on the Serra Geral of Rio Grande do Sul, Brazil. Over 500 frogs and toads of different body size were handled in order to evaluate an improved protocol without narcosis using soft infusion tubes in order to avoid negative effects of the flushing procedure. The specimens were treated soon after capture and then returned to the sampling sites. Our results are discussed with reference to the few studies on stomach flushing in amphibians. The improved technique is recommended to avoid killing of numerous animals for nutritional studies, in particular because of the worldwide threatening of amphibian populations.

Keywords: *Diet, stomach-flushing, Amphibia, Anura, biodiversity, Araucaria forest, Brazil*

Introduction

A major topic in our long-term studies on amphibians on the *Araucaria* plateau of Rio Grande do Sul, Brazil (Kwet, 2001) is the role of anurans as predators and prey in the ecosystem of *Araucaria* rain forests and surrounding habitats (Engels & Heinle, 2003). About 39 species of amphibians have been recorded so far in or near the mountain forest reserve Pró-Mata on the Serra Geral, some of them in high abundance (Kwet, 2004). Dietary patterns in anurans have been commonly analysed by sacrificing the animals, dissecting their stomach and determining the prey remnants (Toft, 1981; Das & Coe, 1994; Flowers & Graves, 1995; Evans & Lampo, 1996; Lima, 1998; Löw & Török, 1998; Anderson et al., 1999; Daza-Vaca & Castro-Herrera, 1999; Peltzer & Lajmanovich, 1999; Van Sluys et al., 2001). In order to avoid killing a representative number of specimens, alternative methods were developed of which the stomach-flushing approach is particularly simple and effective. Originally applied to salamanders (Fraser, 1976) and freshwater turtles (Legler, 1977), the technique was also successfully used in lizards and frogs (Legler & Sullivan, 1979). The treatment was mostly done after narcotising the specimens but this sometimes caused mortality (Joly, 1987). Because amphibians are declining worldwide (Wake, 1991), any research affecting the biodiversity should be scheduled with care.

We used stomach-flushing over four years in a study of the diet of 15 anuran species at the Pró-Mata reserve. Our experience from well over 500 treated frogs and toads resulted in an improved protocol for flushing without anaesthesia of small as well as large individuals, representing all adult stages.

Material and methods

Study area

Our study site was the forest reserve Centro de Pesquisas e Conservação da Natureza Pró-Mata,

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located at altitudes of about 900 m a. s. l. on the Serra Geral in Rio Grande do Sul, Brazil, approximately 150 km northeast of Porto Alegre. The site has a humid subtropical climate.

Sample of anurans

The field studies were carried out from 1999 to 2003. Frogs and toads belonging to five families were collected manually in their habitats and taken immediately to the laboratory for flushing (Table I). A total sample of 583 individuals was stomach-flushed. Most of them were returned to place of capture soon after the procedure, except 15 *Physalaemus lisei*, 4 *Leptodactylus plaumanni* and 10 *Pseudis cardosoi* which were kept in the laboratory for one month and observed for possible harm by the treatment.

Flushing technique

Flushing should be applied as soon as possible after capturing anurans, in order to precede digestion (Secor & Faulkner, 2002). We flushed all animals within three hours of capture. The following equipment (Figure 1) is required for anuran stomach-flushing:

Spatula, forceps, two syringes with thread (20 ml for small, 60 ml for large frogs), infusion tube of soft material (silicon), in particular for small individuals in order to avoid perforations of oesophagus and stomach, with thread, sieve, small airtight vials, two vessels, 70% ethanol. The water for flushing should be taken from the ponds where the anurans were captured and used after filtration. For direct developing animals as the frogs of the genus *Eleutherodactylus*, spring water should be used.

Description of the procedure:

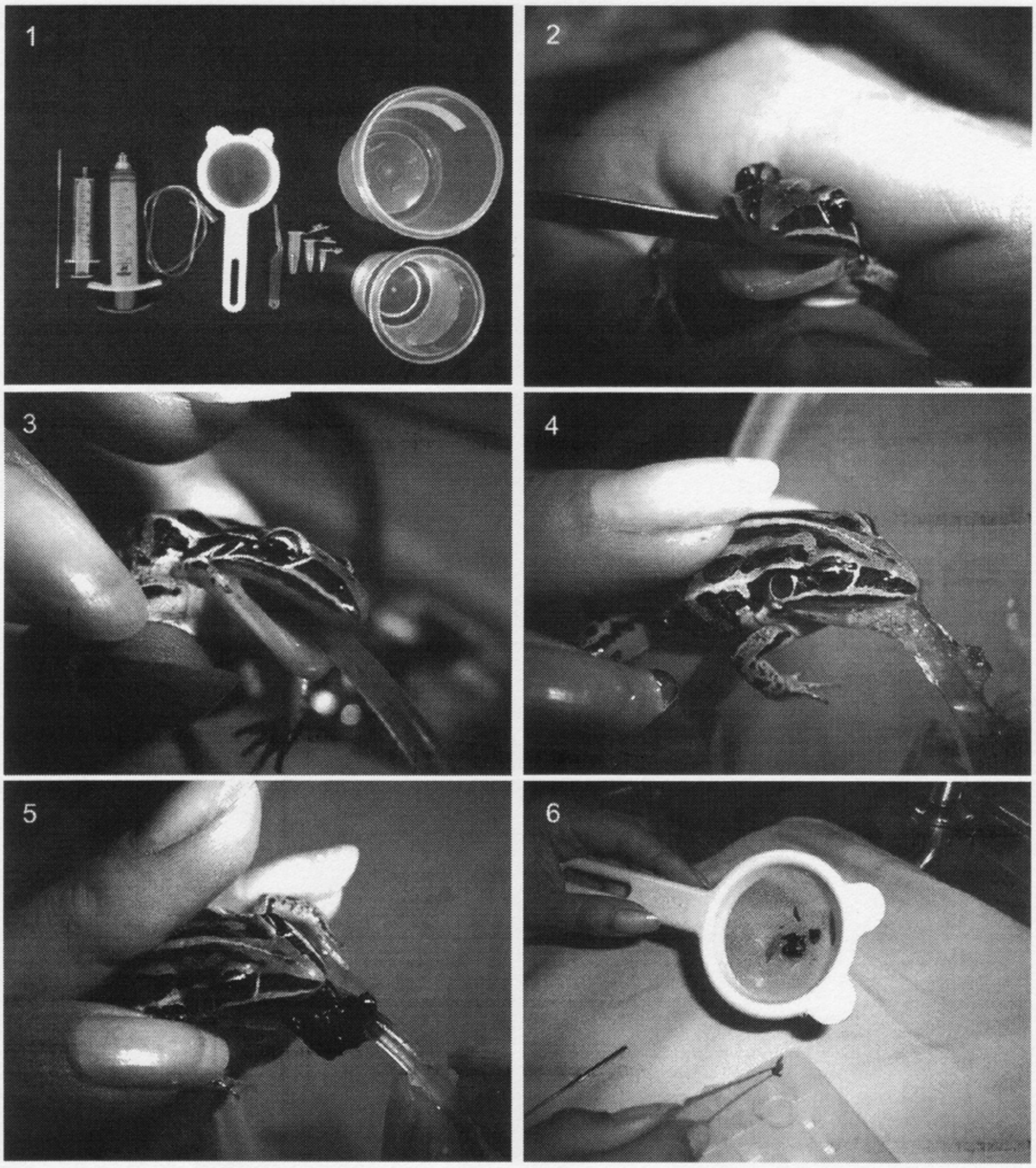
The flushing is done without any anaesthetising and with careful handling of the animals.

Step 1: The anuran can be held safely by fixing the forelimbs with one hand (Figure 2).
Step 2: With the animal in one hand and the prepared syringe (water-filled, tube attached) in the other, the mouth is opened with the help of a spatula. As both hands are in use, the spatula has to be held with the mouth. The tube of the syringe must be introduced through the oesophagus into the stomach. The pyloric end of the stomach can normally be felt (Figure 3).
Step 3: The entire content of the syringe is flushed into the stomach and any content that is forced out is collected in the vessel (Figures 4 and 5). The empty syringe should be separated from the tube, refilled with water and connected to the tube again to repeat the flushing procedure. This procedure should be repeated as long as stomach contents are forced out. When no more stomach content appears, the animal should be flushed one last time.

The water with stomach content is then decanted into a sieve. The items are picked up with forceps and fixed in 70% ethanol in a vial. For smaller specimens, 6 μm gauze is used instead of the sieve.

Table I. Size range and survival of anuran specimens that were stomach-flushed. Minimum and maximum size data correspond to adults and are taken from Kwet and Di-Bernardo (1999).

Family	Species	SVL mm	Flushed	Perished	Survived
Bufonidae	Bufo ictericus	100 – 140	78	1	77
Hylidae	Hyla faber	85 – 100	50	0	50
	Hyla minuta	21 – 28	50	0	50
	Hyla pulchella	33 – 47	50	0	50
	Scinax granulatus	34 – 43	50	1	49
	Scinax perereca	36 – 46	50	0	50
Leptodactylidae	*Eleutherodactylus* cf. *guentheri*	21 – 38	4	0	4
	Hylodes meridionalis	35 – 50	7	6	1
	Leptodactylus plaumanni	35 – 46	43	0	43
	Leptodactylus ocellatus	90 – 120	50	0	50
	Physalaemus cuvieri	24 – 32	8	0	8
	Physalaemus lisei	21 – 33	57	0	57
Microhylidae	Elachistocleis bicolor	20 – 45	16	0	16
	Elachistocleis erythrogaster	29 – 38	10	0	10
Pseudidae	Pseudis cardosoi	34 – 43	60	0	60
	Total		**583**	**8**	**575**



Figures 1 – 6. Flushing technique. (1) Equipment needed for stomach-flushing. (2) Immobilisation of the frog (*Leptodactylus plaumanni*) and insertion of the spatula to open the mouth. (3) Frog with tube entering the mouth (and reaching the stomach). (4) Frog being stomach-flushed. (5) Stomach contents pouring out of the mouth after flushing. (6) Sieve with stomach content to be fixed and stored.

The stomach content can directly be washed with 70% ethanol from the gauze into a small vial.

The subsequent immediate release into their home habitat ensures that the current activity of the treated specimens is not essentially disturbed by the stomach-flushing.

Results

Stomach content obtained by flushing

Because anurans were stomach-flushed within three hours after capture, few of the items were totally intact, most were partially digested. However, parts with heavily sclerotised cuticle remained undigested so that single wings, heads, thorax and abdominal segments of arthropods allowed an identification of the item, at least to order level.

The presence or absence of animal food, which could be recovered through stomach-flushing, varied depending on the analysed anuran taxa. In some species all flushed animals had stomach contents. This was the case for the leptodactylid frogs *Eleutherodactylus* cf. *guentheri*, with small arthropods in the stomach, *Hylodes meridionalis*, with mainly dipterans and coleopterans as stomach content, *Leptodactylus ocellatus*, preying on large coleopterans, spiders and other anurans, and *Physalaemus cuvieri*, with mostly small arthropods in the stomach and for the microhylids *Elachistocleis bicolor* and *E. erythrogaster*, both feeding exclusively on ants and termites. In 90% of the *Pseudis cardosoi*, food items were recovered, consisting mostly of coleopterans, dragonfly nymphs and hymenopterans. The animals with empty stomachs were captured during cold nights and therefore were probably not hunting. 88% of the *Physalaemus lisei* revealed stomach contents consisting mainly of ants, dipterans and spiders. From 77% of the *Leptodactylus plaumanni* food was recovered, consisting mostly of coleopterans and ants. Only half of the *Bufo ictericus* had food remnants, with coleopterans, ants and diplopods being the most frequent taxa. The other half were males with empty stomachs caught at a pond at night while calling. Male *Bufo ictericus* seem to stop feeding during courtship. Something similar seems to happen with the tree frogs: of the five species examined only *Hyla pulchella* revealed a high number of animals with food items in their stomachs, mainly being coleopterans, lepidopterans and hymenopterans. Of the two examined species of the genus *Scinax* only about 35% had stomach contents, those being mostly homopterans, coleopterans and mites for *Scinax granulatus* and spiders and blattarians for *Scinax perereca*. Nearly all smith-frogs (*Hyla faber*) had empty stomachs. The few frogs with stomach contents had mostly preyed on opilionids and anurans.

The largest prey items were a male *Scinax granulatus* (36.2 mm SVL) eaten by a male *Hyla faber* (95.3 mm SVL), a mygalomorph spider with a prosoma measuring 20.6 mm eaten by a female *Leptodactylus ocellatus* (69 mm SVL) and a 48.2 mm large dragonfly nymph captured by another female *L. ocellatus* (78.6 mm SVL). The smallest items were collembolans and mites.

Reliability of the flushing technique

Flushing is a reliable method to recover stomach contents. In most of the anurans the contents were recovered completely after the second flushing and only seldom did the procedure need to be repeated (maximum of six times) before all contents were recovered. If individuals are captured during or shortly after their foraging activity and flushed no longer than three hours later most of the stomach contents will be undigested and can subsequently be identified without problems.

Survival of the flushed anurans

Nearly all flushed anurans survived the procedure without apparent problems. Of eight injured specimens, one *Bufo ictericus* and one *Scinax granulatus* died because their gut was punctured with the tube and the lungs were filled with water. Six *Hylodes meridionalis* perished without apparent harm, showing strong muscular contractions in the hind limbs shortly before death. These specimens, analysed in the initial phase of our study, had been flushed with tap water. Without our knowledge this had been treated with high doses of chlorine and this fact presumably caused the death of the frogs. Therefore, we decided to use only pond or spring water to flush the stomachs. Altogether the total mortality in our experiments was about 1% only.

Twenty-nine stomach-flushed frogs were kept in captivity for one month and accepted termites as food within the next two hours. After one month, they all seemed to be healthy and were released back to the pond where they had been collected.

Discussion

Evaluation of the improved stomach-flushing technique

Our flushing protocol differs from previous methods. The whole procedure can be conducted by a single person. No anaesthetic is needed which caused mortality in previous studies (Legler & Sullivan, 1979). The use of a soft silicone infusion tube prevents lesions of the gut tissues. Even small and recently metamorphosised frogs with an SVL of only 21 mm can be flushed without problems. The immediate return to the place of capture prevents behavioural disturbance, especially during the period of reproduction.

Previous experience with flushing of amphibians

Since introduction of the stomach-flushing method (Legler & Sullivan, 1979) with brevital sodium to anesthetise animals, the technique was modified in subsequent studies. Working with ranid frogs, Leclerc and Courtois (1993) used tricaine methane sulphonate as a narcotic in order to reduce the mortality. These authors killed some flushed frogs to test if food remained in the stomach and found that less than 1% of the total food volume was not removed. Stomach-flushing was used by Ovaska (1991) to analyse the diet of *Eleutherodactylus johnstonei* in Barbados and by Díaz-Páez and Ortiz (2003) to study the feeding habits of the leptodactylid frog *Pleurodema thaul* in Chile. Lamoureux et al. (2002) used stomach-flushing in order to study premigratory autumn foraging forays in the green frog, *Rana clamitans*. Tocque et al. (1995) studied the prey range of the desert toad (*Scaphiopus couchii*) but did not precisely describe their stomach-flushing method. In some European anurans this technique was used to determine the minimum sample size for estimating diet diversity (Kovács & Török, 1997). Gittins (1987) compared the results of stomach-flushing in common toads (*Bufo bufo*) with dissections of road casualties and obtained similar results. Patto (1998) adapted stomach-flushing to small frogs working with *Hylodes asper*. He anaesthetised them for a few seconds in a plastic bag containing cotton soaked with chloroform before flushing the stomach. He subsequently dissected six animals and detected no food remains. Of 97 toe-clipped and released frogs 13 were recaptured once and seven twice. They had food in their stomachs, suggesting that they had not been injured by the procedure and had resumed normal feeding activities. Patto also tested the method on an abundant invader species, *Leptodactylus fuscus*. Of the 20 flushed and the dissected frogs, none had any more food items in the stomach. He recommended the stomach-flushing approach in dietary studies of rare, endemic, or protected species and for research on Amphibian population dynamics.

Conclusions

Our improved stomach-flushing protocol was tested with small (SVL 21 mm) and large anurans (SVL 140 mm). The low proportion of injured individuals showed that this method works well if correctly applied. The fact that 29 stomach flushed frogs survived well and fed normally in captivity suggests that they suffered no inner lesions or other disturbances during the procedure. Corresponding results were obtained in the first application of our method for dietary studies in the microhylid frog *Elachistocleis bicolor* (Solé et al., 2002). Therefore, we can strongly recommend using stomach-flushing in research on the diet of anurans. We agree with the statement of the Northern Territory University Animal Experimentation Ethics Committee that 'there is now no justification for killing animals solely in order to obtain stomach samples' (Northern Territory University, 2000). In addition, the programs of monitoring biodiversity and the nutritional requirements of vertebrate species should also consider these aspects.

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5.3. **Solé, Mirco**[1]; Pelz, Birgit[1]; Di-Bernardo, Marcos[2] & Kwet, Axel[3]

Do male tree frogs feed during the breeding season? A Comparison of five hylid species in Rio Grande do Sul, southern Brazil.

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Da die meisten Laubfrösche auf Wasseransammlungen angewiesen sind, um sich fortpflanzen zu können, müssen sie ihre arborealen angestammten Lebensräume verlassen. Die Fortpflanzungszeit- und dauer variiert bei den verschiedenen Arten und ist klimaabhängig. Wir untersuchten die Ernährung von fünf Laubfroscharten mit unterschiedlichen Fortpflanzungsstrategien. *Hyla faber* (Hauptrufzeit 2 Monate im Jahr), *Hyla* cf. *minuta* (4 Monate), *Hyla pulchella* (8 Monate), *Sinax granulatus* und *S. perereca* (3 Monate). Es wurden jeweils 50 rufende Männchen gefangen und deren Mageninhalte mittels einer Magenspülungsmethode untersucht. Die Hälfte der untersuchten Frösche hatte Nahrungsreste im Magen. In 18-34 % der Mägen fanden wir pflanzliche Reste, die wahrscheinlich aus Versehen mitverzehrt wurden. Sie verbleiben lange Zeit unverdaut im Magen. Während wir nur in 8% der Mägen von *Hyla faber*, 24% der Mägen von Hyla cf. *minuta*, 36 % der Mägen von *Scinax granulatus* und 34 % der Mägen von *Scinax perereca* tierische Nahrungsreste fanden, hatten 84 % der untersuchten

Hyla pulchella solche Beute im Magen. Es konnte eine Korrelation zwischen einer kurzen Fortpflanzungsperiode und einer reduzierten Nahrungsaufnahme während dieser Zeit sowie einer langen Fortpflanzungsaktivität und regelmäßiger Aufnahme von Nahrung festgestellt werden. *Hyla pulchella* ist außerdem der einzige untersuchte Laubfrosch, der auch außerhalb der Fortpflanzungsperiode auf offenen Grasflächen in der Nähe seiner Fortpflanzungsgewässer lebt, während die Frösche der anderen untersuchten Arten nach ihrer Fortpflanzungsaktivität wieder Waldgebiete aufsuchen.

Do Male Tree Frogs Feed During the Breeding Season? A Comparison of Five Hylid Species in Rio Grande do Sul, southern Brazil.

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Introduction

Many tree frogs (Hylidae) have to leave their actual habitats for mating, because reproduction of hylids is usually linked to water (DONNELLY & GUYER, 1994). For that reason they may cover some distances to move to suitable water bodies. The duration of breeding season differs among species and depends on factors like climate and reproductive behaviour. The subtropical Northeast of Rio Grande do Sul, Brazil, provides many different habitats for anurans and shows a high diversity of hylid species (29 of the 71 recorded species are tree frogs) (KWET, 2001) with differing reproductive patterns, from prolonged breeders to explosive breeders.

Mating activity is associated with high costs, especially for males: calling increases not only the risk of becoming prey, but also rises energy consumption. However, focussing on diet to compensate the energy loss would minimize the success of mating efforts. Hence males of species with short reproductive periods may even be able to stop feeding for that period. Therefore there might be a correlation between feeding behaviour and duration of breeding season.

Materials and methods

We have chosen five hylid species with different reproductive patterns: *Hyla faber* (main calling activity about two months per year (MARTINS, 1993)), *Hyla* cf. *minuta* (about four months per year), *Hyla pulchella pulchella* (about eight months per year), *Scinax granulatus* and *Scinax perereca* (each about three months per year) (KWET, 2001; KWET & DI-BERNARDO, 1999).

For each species the stomach contents of 50 male specimens were examined. The frogs were collected at the CPCN Pró-Mata, located at the Serra Geral of Rio Grande do Sul during their reproductive period in spring and early summer (November 2002 to January 2003) at their typical calling sites, usually at night. Their stomachs were emptied no more than three hours after the capturing of the frog by stomach flushing (PATTO, 1998, SOLÉ *et al.*, 2002; SOLÉ *et al.*, in press.), a simple and frog-sparing method. After that procedure the animals were released. The stomach content was preserved in 70% ethanol and later identified under a binocular.

Results

Half of the examined frogs had empty stomachs, but there were differences between the species: The number of stomachs with content ranged from 19 (*Hyla faber*) to 42 (*Hyla pulchella pulchella* (fig.1). The $\chi 2$-test shows a significant difference ($p = 0.01$).

The stomach content was composed of animal prey items and/or remains of plants (fig. 1). The numbers of stomachs containing animal items were obviously dispersed among species (fig. 2). The $\chi 2$-test shows a significant difference ($p = 0.001$). The few animal preys ingested by adult male *Hyla faber* during their breeding season were Opiliones, larval Lepidoptera and other anurans. *Hyla minuta* fed mostly on Aranaeae, Diptera and Homoptera. *Hyla pulchella pulchella*, the only studied species with a regular intake of animal food-items fed mostly on Coleopteran, larval Lepidoptera and Hymenoptera. Coleoptera and Homoptera were the animal taxa mostly ingested by *Scinax granulatus*. *Scinax perereca* fed on Araneae and Blattaria.

Quite frequently we found remains of plants in the frogs' stomachs. That was observed likewise for all species (fig. 3) and did not show any significant difference ($\chi 2$-test, $p = 0.7$).

To examine a correlation between the duration of breeding season and the number of stomachs containing prey items, the Spearman-rank-correlation-coefficient for these parameters was calculated. It shows a significant correlation ($p = 0.05$).

Discussion

The assumed correlation between the duration of breeding season and feeding behaviour could be demonstrated among the observed hylid species: the longer the reproductive period, the more male frogs do feed during that time.

Plant remains were evenly distributed in the stomachs of all species. Presuming that plants are occasionally devoured along with prey, but remain in the stomach for quite a long time due to lack of digestion, these remains might be no indicator for actual, i.e. recent feeding.

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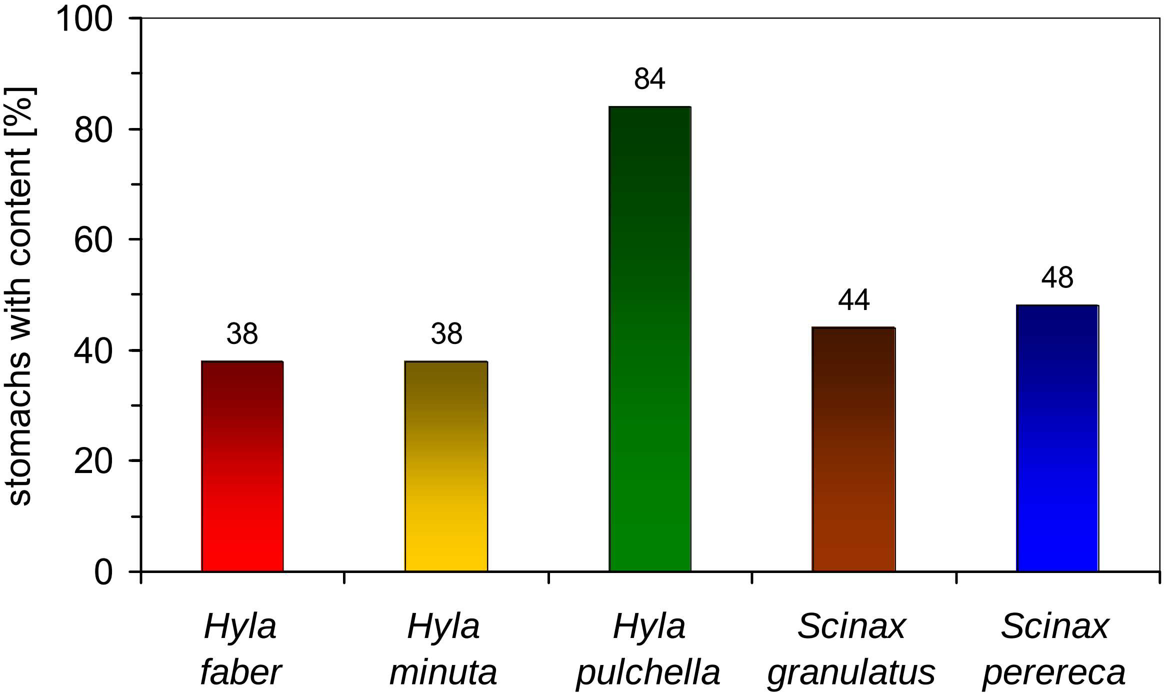

Fig. 1: Percentage of stomachs with content
n = 50 for each species

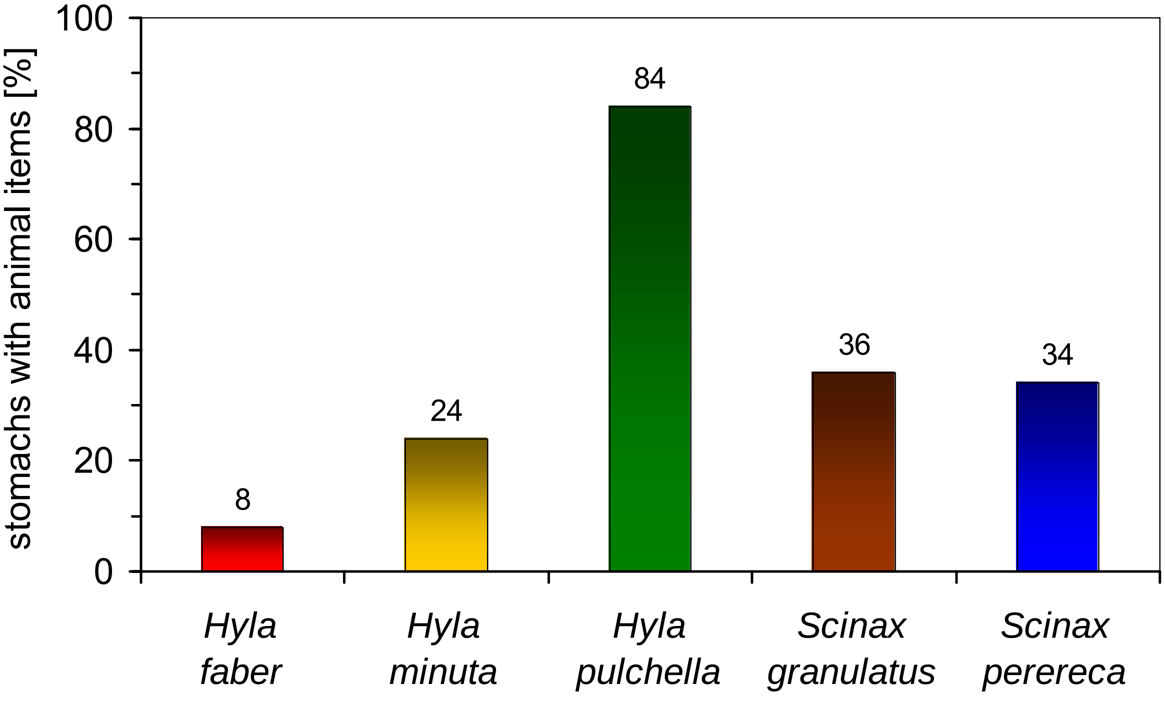

Fig. 2: Percentage of stomachs containing animal prey items
n = 50 for each species

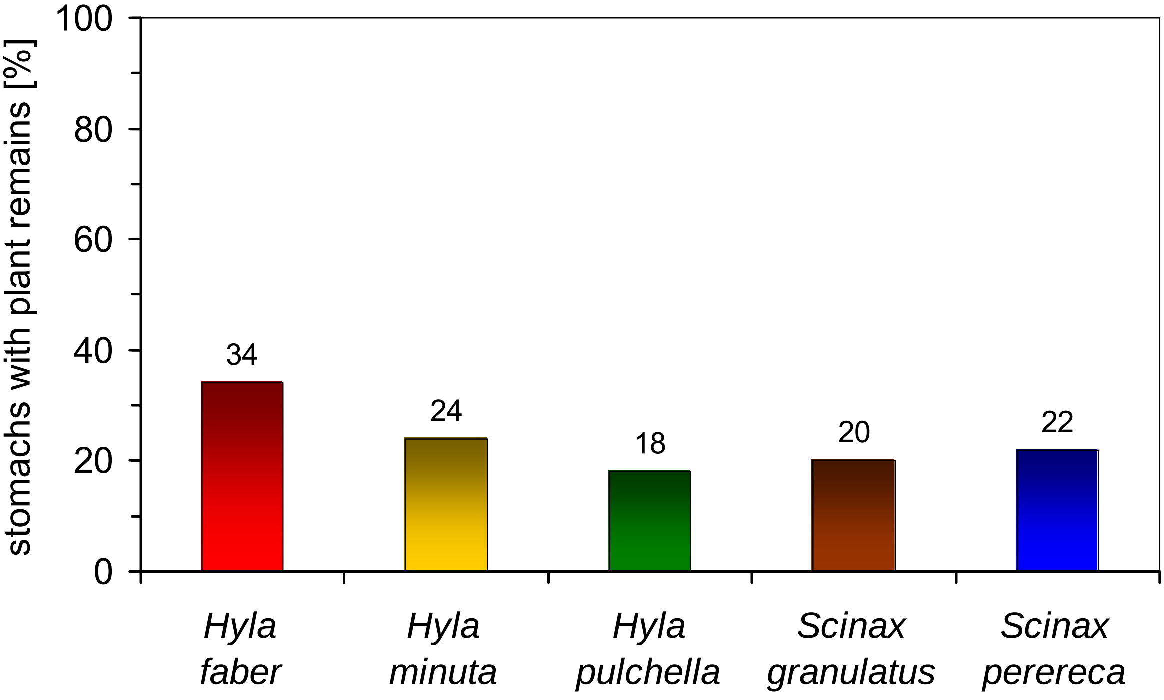

Fig. 3: Percentage of stomachs containing plant remains
n = 50 for each species

	Hyla faber	*Hyla minuta*	*Hyla pulchella*	*Scinax granulatus*	*Scinax perereca*
n examined stomachs	50	50	50	50	50
n stomachs with plant remains	17	12	9	10	11
n stomach with animal items	4	12	42	18	17
n found animal items	12	28	148	30	32
Acari		1	3	3	
Anura	2				
Araneae		13	9	1	10
Blattaria	1		2		8
Coleoptera	1	2	66	4	2
Collembola			1		
Dermaptera			1		
Diptera		4	10	2	
Homoptera		3	8	4	2
Hymenoptera		1	12	2	
Isoptera				2	
Lepidoptera (larval)	2		13	1	2
Lepidoptera (adult)			5		
Odonata			3		
Opiliones	3		2		
Orthoptera			1	1	
not identified	3	4	12	10	8

Tab. 1: stomach contents
List of animal prey items found in the stomachs of 250 male hylids

5.4. Kwet, Axel[1] & **Solé**, **Mirco**[2]

Taxonomic status of *Hylodes henselii* Peters, 1870 from southern Brazil, and description of acoustic variation in *Eleutherodactylus guentheri* (Anura: Leptodactylidae).

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Die taxonomische Stellung vieler im Araukarienwald lebender Frösche ist nicht ausreichend geklärt. Wir untersuchten die Anzeigerufe der Männchen von *Eleutherodactylus guentheri* aus verschiedenen Lokalitäten in Südbrasilien. Aufgrund signifikanter Unterschiede in den Lauten schließen wir, dass es sich bei der südlichen Form um eine eigenständige Art handelt. Für diese ist der Name *Hylodes henselii* Peters, 1870 verfügbar. Der lange Zeit als verloren geglaubte Holotyp dieser Art wurde im Zoologischen Museum Berlin wieder entdeckt. *Eleutherodactylus henselii* ist innerhalb seiner Gattung die Art, die in der Neotropis am weitesten in den Süden vordringt: Er kommt im subtropischen Atlantischen Regenwald und im Araukarienwald der Staaten Rio Grande do Sul und Santa Catarina, außerdem in Misiones, Argentinien vor. Die bioakustischen Daten weisen darauf hin, dass sich offensichtlich in der *Eleutherodactylus guentheri* - Gruppe mindestens eine weitere kryptische Art verbirgt.

Title Page

JOURNAL OF HERPETOLOGY

LRH: A. Kwet and M. Solé

RRH: Taxonomic status of *Hylodes henselii*

Taxonomic status of *Hylodes henselii* Peters, 1870 from southern Brazil, and description of acoustic variation in *Eleutherodactylus guentheri* (Anura: Leptodactylidae)

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Key Words: Leptodactylidae, taxonomy, Rio Grande do Sul, *Eleutherodactylus henselii, Eleutherodactylus guentheri,* sibling species, advertisement call, *Eleutherodactylus manezinho*

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Taxonomic status of *Hylodes henselii* Peters, 1870 from southern Brazil, and description of acoustic variation in *Eleutherodactylus guentheri* (Anura: Leptodactylidae)

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ABSTRACT.—The comparison of advertisement calls of *Eleutherodactylus guentheri* from different localities in southern and southeastern Brazil revealed considerable acoustic variation between populations. Due to significant differences in call parameters, we conclude that the southern form must be considered a distinct species, for which the name *Hylodes henselii* Peters, 1870 is available. An examination of the holotypes of *Hylodes henselii* and *Hylodes guentheri* Steindachner, 1864, along with recently collected material, showed that both species are morphologically very similar, being thus sibling species. *Eleutherodactylus henselii* is the southernmost member of the genus *Eleutherodactylus* occurring in subtropical Atlantic rain forest and *Araucaria* forest of the Brazilian states of Rio Grande do Sul and Santa Catarina, and Misiones, Argentina. The bioacoustic data suggest that at least another cryptic species is involved in the *Eleutherodactylus guentheri* group.

Hylodes henselii Peters, 1870 is currently considered a synonym of *Eleutherodactylus guentheri* (Steindachner, 1864) (Heyer, 1984; Frost, 2002), a wide-spread leptodactylid occurring in the Atlantic forest and *Araucaria* forest morphoclimatic domains of southern and southeastern Brazil, ranging from northeastern Rio Grando do Sul to Espirito Santo (Heyer, 1984), in Misiones province, Argentina (Carrizo et al., 1989; Lavilla & Cei, 2001), and presumably in adjacent Paraguay (Frost, 2002). Lynch (1976) revised the species groups of *Eleutherodactylus* (*Hylodes* sensu Peters, 1870) and recognized a *binotatus* series, in which he tentatively included *E. henselii* as a valid species, among other taxa, like *E. binotatus* and *E. guentheri*. Heyer (1984) doubted the validity of this *binotatus* group and proposed a monophyletic *guentheri* cluster. He regarded *Hylodes henselii* as a synonym of *E. guentheri* and followed Lynch (1976) in considering the holotype of *henselii* to be lost. Heyer (1984) made an arbitrary decision to clarify the nomenclature by designating the holotype of *guentheri* (NHMW 16515) as the neotype of *henselii*. In the most recent classification as proposed by Lynch and Duellman (1997) and Lynch (2001), the *guentheri* cluster of Heyer (1984) is allocated within a broadly interpreted *Eleutherodactylus binotatus* series.

The holotype of *Hylodes henselii* (ZMB 6813) collected near Porto Alegre, Rio Grande do Sul, southern Brazil, was recently recovered in the collection of the Zoological Museum Berlin (Bauer et al., 1995). We compared this specimen with material identified as *E. guentheri* from other collections. Additionally, we analyzed advertisement calls assigned to *E. guentheri* from various localities in Brazil and Argentina. Due to significant differences in many parameters, like call length, number of notes per call and note repetition rate, we conclude that the southern form must be considered a distinct species, for which the name *Hylodes henselii* Peters, 1870 is available and should be revalidated. The purpose of this paper is to resurrect *Eleutherodactylus henselii* and to describe the acoustic variation in *E. guentheri*.

MATERIAL AND METHODS

The following measurements were taken to the nearest 0.1 mm with calipers: snout-vent length (SVL); head length, defined as the diagonal distance from the tip of the snout to the right angle of the jaw (HL); greatest head width (HW); horizontal eye diameter (ED); horizontal tympanum diameter (TD); interorbital distance (IO); internarial distance (IN); eye-nostril distance (EN); thigh length (TL); shank length (SL); foot length (FL). Specimens examined are deposited in the following collections: Biogeographische Sammlung der Universität des Saarlandes (BGSS, Saarbrücken, Germany), Centro Nacional de Investigaciones Iológicas (CENAI, now in the MACN collection), Museo Argentino de Ciencias Naturales (MACN, Buenos Aires, Argentina), Museu de Ciências e Tecnologia da PUCRS (MCP, Porto Alegre, Brazil), Museo de Ciencias Naturales de La Plata (MLP, La Plata, Argentina), Museu Nacional Rio de Janeiro (MNRJ, Rio de Janeiro, Brazil), Naturhistorisches Museum Wien (NMW, Wien, Austria), Staatliches Museum für Naturkunde Stuttgart (SMNS, Stuttgart, Germany), National Museum of Natural History (USNM, Washington D.C., USA), and Museum für Naturkunde Berlin, formerly Zoologisches Museum Berlin (ZMB, Berlin, Germany).

Most recordings were obtained with a Sony WM-D6C tape recorder and a Sennheiser microphone (module ME66, powering module K6). The calls were analyzed with a Macintosh-based signal analysis software (Canary 1.2, Cornell University) at a sampling frequency of 44.1 kHz. Measurements of the following acoustic parameters were taken: call duration, note duration, note interval, notes per call, note repetition rate, dominant frequency, and other frequencies. Temporal parameters were measured in seconds (sec) or milliseconds (msec) and spectral parameters in Hertz (Hz). Tape recordings of morphologically identified *E. guentheri* (five calls per individuum when available) and *E. manezinho*, which occurs syntopically, were analyzed from the following locations (arranged from South to North):

Brazil: Rio Grande do Sul: São Francisco de Paula: Centro de Pesquisas e Conservação da Natureza Pró-Mata; recorded by AK, (a) voucher specimen SMNS 9005, 9 November 1995, 0030 h, 19 C air temperature; (b) voucher specimen MCP 3182, 7 December 1997, 2330 h, 19 C air temperature.

Brazil: Santa Catarina: Florianópolis: Ilha de Santa Catarina; recorded by AK, unvouchered, 11 October 2002, 2130 h, 20.5 C.

Brazil: Santa Catarina: Ilhota: Morro do Baú; recorded by AK, unvouchered, 23 October 2002, 1000 h, 17.5 C.

Brazil: Santa Catarina: Guarámirim: Instituto Rã-bugio; recorded by G. Woehl, three different calls (available at http://www.ra-bugio.org.br/index.html), unvouchered, without data.

Brazil: Santa Catarina: Pirabeiraba; recorded by W. R. Heyer, voucher specimen USNM 235727, 10 December 1978, 2030 h, without temperature record.

Brazil: São Paulo: Serra da Bocaina; recorded by W. R. Heyer, voucher specimen USNM 235699, 23 December 1976, 2000-2145 h, 19-20 C.

Brazil: São Paulo: Boracéia; recorded by A. S. Rand, two unvouchered specimens, 9 December 1984, 1920 h, 19.2 C.

Argentina: Misiones: Departamento San Pedro: Colonia Victoria; recorded by D. Baldo, two voucher specimens MLP DB 1923-24, 14 October 2002, 2205-2220 h, 19-20 C.

E. manezinho, Santa Catarina: Florianópolis: Ilha de Santa Catarina; recorded by AK; (a) voucher specimen MCP 6365, 6 October 2002, 2230 h, 20.5 C; (b) voucher specimen MCP 6300, 17 November 2003, 2230 h, 23 C.

RESULTS

Eleutherodactylus henselii (Peters, 1870)

Figs. 1-3

Hylodes sp. (*Cystignathus* sp.).—Hensel, 1867:161-162.

Hylodes henselii.—Peters, 1870:648 (holotype: ZMB 6813; type locality: near Porto Alegre, Rio Grande do Sul, Brazil); Bokermann, 1966:67 (partim).

Hylodes griseus (non Hallowell).—Boulenger, 1882:206 (partim), 1886:440; Nieden, 1923:460 (partim).

Hylodes gollmeri (non Peters).—Baumann, 1912:143, 150, 161 (partim).

Hylodes ranoides (non Spix).—Miranda-Ribeiro, 1926:53-55 (partim).

Eleutherodactylus griseus (non Hallowell).—Gorham, 1966:75-76 (partim).

Eleutherodactylus henselii.—Lynch, 1976:3, 7; Harding, 1983:162.

Eleutherodactylus guentheri (non Steindachner).—Cochran, 1955:271-274 (partim); Lynch, 1976:7 (partim); Braun & Braun, 1980:132; Heyer, 1984:1-42 (partim); Carrizo et al., 1989:10-11; Lavilla, 1992:83; Bauer et al., 1995:46; Garcia, 1996:57-68 (partim); Lavilla & Cei, 2001:105-106; Lavilla et al., 2002:106-107.

Eleutherodactylus cf. *guentheri*.—Kwet, 1999:97-98; 2001:33, 35, 189; Kwet & Di-Bernardo, 1999:60-61.

Diagnosis.— *Eleutherodactylus henselii* is a medium sized species of the *Eleutherodactylus guentheri* cluster sensu Heyer (1984). It is perfectly characterized by the very prolonged advertisement call with a duration of about 10-25 seconds, consisting of a series of more than 80 notes (up to 170). In other species of the *guentheri* cluster the call is much shorter, consisting of 5-10 notes, with maximum of 35 with a duration of less than three seconds. *Eleutherodactylus henselii* is a cryptic species being morphologically not absolutely distinguishable from *E. guentheri* (for description of *E. guentheri* from the type locality see Cochran, 1955). Whereas *E. henselii* has a rounded, slightly protruding snout profile (Figs. 2A, B), the snout is more sloping in *E. guentheri* (Fig. 4B). The throat is usually white and unmottled in *E. henselii*, whereas gray mottled in *E. guentheri*. Other *Eleutherodactylus* species, occurring syntopically with *E. henselii* are *E. binotatus* and *E. manezinho*. *Eleutherodactylus henselii* is distinguished from the larger *E. binotatus* by lacking of dorsal longitudinal skin crests and the first finger possessing about the same length as the second (first finger much longer than second in *E. binotatus*). It is distinguished from *E. manezinho* by the more slender body, smaller finger discs and longer hindlegs (shank length about 68-69% SVL in *E. henselii*; 58% SVL in *E. manezinho*; Garcia, 1996).

Description.—This redescription is based on all available specimens from Rio Grande do Sul and southern Santa Catarina which are, due to their advertisement calls, referrable to *E. henselii* (see Appendix 1). Measurements (Table 1) and ratios are only from specimens which sex could be determined. *E. henselii* is characterized by a distinct sexual dimorphism (SVL 21.0-27.5, mean 24.3 mm in males; 28.4-38.4, mean 33.4 mm in females). Measurements (mm) for the holotype, ZMB 6813, an adult female faded and in poor condition (Figs. 1, 2A), are as follows (in mm): SVL 32.5, HL 12.7, HW 11.4; ED 3.6; TD 2.0; EN 3.8; IO 5.6; IN 3.0; TL 20.6; SL 23.4; FL 19.8. Head longer than wide, its length 42.3% SVL in males, 41.6% in females, its width 34.6% SVL in males, 35.3% in females. Snout rounded, protruding in profile (Figs. 2A, B), subovoid in dorsal view. No cranial crests, canthus rostralis evident, loreal region flat and nearly vertical. Nostrils not protuberant, much closer to tip of snout than to eye. Eyes large, their diameter about 25% head length. Upper eyelids smooth or with small tubercles. Tympanum distinct, its diameter 55-60% eye diameter. Slight supratympanic ridge. Vocal sac small, single, subgular. Vocal slits present in males. Vomerine teeth in two short, narrowly separated, transverse rows, located behind and between the choanae. Skin on dorsum smooth or indistinctly granulated, usually with two weak dorsolateral glandular folds and a Y- or W-shaped glandular ridge between the shoulders. Skin on throat and venter smooth. Limbs slender, hindlegs elongated in proportion to body. Thigh length on average 61.3% SVL in males, 62.0% in females; tibia length 67.9% SVL in males, 69.2% in females; and foot length SVL 60.1% SVL in males, 60.8% in females. Fingers lacking webbing and lateral fringes but possessing fine dermal ridges laterally. Finger discs distinct, their width about 1.2-1.5 times finger width. Relative lengths of fingers 1≈2<4<3. Subarticular tubercles distinct, rounded and moderately raised. Large, oval, moderately raised outer and inner metacarpal tubercles (Fig. 2C). Indistinct, cream nuptial asperities on the inner base of the first finger in males. Toes not webbed, laterally fringed. Relative lengths of toes 1<2<5<3<4. Foot texture smooth with rounded, raised subarticular tubercles. Inner metatarsal tubercle elliptical, protruding; outer metatarsal tubercle small, rounded (Fig. 2D). Tarsal fold absent.

Color in life.—Color photos of *Eleutherodactylus henselii* (as *Eleutherodactylus* cf. *guentheri*) are in Kwet (1999: 97, 2001:35) and Kwet and Di-Bernardo (1999: 61). Dorsal coloration extremely variable, reddish, orange, grayish- or yellowish-brown, uniform or with symmetrical pattern of indistinct dark markings and lines (Figs. 3A-D). Some specimens with white mid-dorsal pin stripe or broad line. Dorsal region of the snout usually light brown, contrasting with the darker brown coloration on loreal region and dorsum. Distinct pattern of dark and light brown vertical bars on upper lip; below eye always a dark broad vertical bar. With or without dark interorbital stripe or bar. Black triangular blotch above tympanum, sometimes posteriorly extended to the groin. Usually two small black inguinal blotches. Hindlegs brownish with 3-5 dark cross-bars. Forearms with a few indistinct brown blotches. Belly and throat dirty yellow or white, unmottled. Posterior surface of thigh yellowish, uniform or indistinctly marbled with brown. Outer shank usually with broad dark stripe, sometimes incomplete or interrupted. Coloration in preservative about the same.

Variation.—The coloration varies as described above. Most specimens are uniformly dark brown to gray or have a symmetrical dorsal pattern of dark markings. Four (MCP 3182, 3183, 3683, 6853, SMNS 9125) out of 42 specimens from Rio Grande do Sul (about 10%) have a broad, distinct, light mid-dorsal stripe, but most specimens are without such a stripe. Skin structure varying from smooth to sligthly tuberculate.

Vocalization.—We recorded two different calls of *E. henselii* (Table 2; Fig. 5). The prolonged call type (Figs. 5A, 6C; presumably the advertisement call) was a sporadically given, castagnette-like sound beginning quietly, rising in intensity and reaching the maximum energy in the last third of the call. The whole call lasted 10-23 sec and consisted of 86-170 unpulsed notes (each note 3.5-6 msec in duration). Note intervals were larger at the beginning of the call (about 130-230 msec), decreasing towards the end of the call (70-130 msec). Accordingly, the note repetition rate increased from 5-6 notes per sec to 8-9 notes per sec at the end of the call. The second call type recorded (Figs. 5B, 8C; presumably an aggressive call) lasted 0.5 sec and consisted of 5-6 notes with a higher note repetition rate of 10-12 per sec. Both call types had a dominant frequency range from 2100 to 3100 Hz and presented a weak harmonic structure; another frequency with perceptable energy ranged from 4000 to 4900 Hz. There was no frequency modulation.

Call variation.—Small differences were revealed when comparing this advertisement call of *E. henselii* from northeastern Rio Grande do Sul (recorded at 19 C air temperature) with the call from Ilha de Santa Catarina which had (at 20.5 C air temperature) on average a shorter call duration and shorter note intervals (Table 2; not figured). However, these differences could be due to temperature effects (temporal parameters depend on air temperature; Duellman and Trueb, 1986) and we refer all citations from southern Santa Catarina (i.e., Ilha de Santa Catarina, Rancho Queimado, Águas Mornas) to *E. henselii*. A recorded call from Guaramirím, northern Santa Catarina (Fig. 6A; available as *Eleutherodactylus* sp1a. at http://www.ra-bugio.org.br/index.html), and a recording from Misiones, Argentina (Fig. 6B), fit the character states of *E. henselii* and definitely belong to this species. On the other hand, all other calls which are currently assigned to *Eleutherodactylus guentheri* differ significantly from this advertisement call and there is also considerable variation between different populations (Table 2; Figs. 7, 8). It seems that at least one or two other cryptic species are involved in the *guentheri* group.

The advertisement call from Serra da Bocaina (Figs. 6D, 7A), which lies near the type locality of *E. guentheri* (about 150 km from Rio de Janeiro), should actually originate from a male of this species. It is considerably shorter (on maximum 1.8 sec) than the advertisement call of *E. henselii* consisting of only 11-36 notes with short note intervals of about 40 msec and much higher note repetition rates of 19-20 per sec. According to Cochran (1955), the call of *E. guentheri* from Tijuca (Rio de Janeiro) is a "sort of quacking, suggesting that of a duck" but this statement is obviously erroneous. In northern Santa Catarina there are several recordings that differ from the calls of Serra da Bocaina. The 2-3 sec lasting call from Morro do Baú (Fig. 7B) seems to be identical with the published call from Pirabeiraba (Heyer, 1984) and a call from Guaramirím (Fig. 7C; available as *Eleutherodactylus* sp25. at http://www.ra-bugio.org.br/index.html). Two different, shorter calls recorded from the same localities in Pirabeiraba (Fig. 8A) and Guaramirím (Fig. 8B; available as *Eleutherodactylus guentheri* at http://www.ra-bugio.org.br/index.html) could be aggressive calls of the same species because they are very similar to the aggressive call of *E. henselii* (Fig. 8C). However, it seems that all these vocalizations are not identical with the advertisement call of *E. guentheri* from Serra da Bocaina, which has a much higher note repetition rate.

Another distinct call is that from Boracéia, São Paulo (Heyer et al., 1990), which differs substantially in several temporal and spectral characteristics (Table 2; Fig. 8D), i.e., by having a longer note duration (each note lasts 10-25 msec consisting of 1-2 pulses) and a relatively high dominant frequency, showing notable frequency modulation (change in dominant frequency from about 2.2 kHz at the beginning of the call to 3.2 at the end of the call). Calls of *E. henselii* and *E. guentheri* are not frequency modulated and have short (3-6 msec lasting), always unpulsed notes. It is remarkable that these unvouchered recordings from Boracéia are nearly identical to the call of *Eleutherodactylus manezinho* from Ilha de Santa Catarina (Fig. 8E; see also Castanho and Haddad, 2000). However, further data are needed to make taxonomic alterations and pending further studies all remaining populations should be named *Eleutherodactylus guentheri*.

Geographical distribution.— *Eleutherodactylus henselii* occurs in the subtropical Atlantic rain forest and parts of the *Araucaria* forest domain in southern Brazil and Misiones, Argentina, at altitudes from about sea-level up to 1200 m. All bibliographical citations for *Eleutherodactylus guentheri* from northeastern Rio Grande do Sul, i.e., municipalities Cambará do Sul, Canela, Caxias do Sul, Gramado, Maquiné, São Francisco de Paula, São Leopoldo, Terra de Areia, and Torres (Braun and Braun, 1980; Heyer, 1984; Kwet & Di-Bernardo, 1999; Kwet, 2001), refer to *E. henselii*, but the species is not known from Porto Alegre as stated in Bauer at al. (1995) and Frost (2002). The nearest recordings are from about 100 km northeast of Porto Alegre. All citations of *Eleutherodactylus guentheri* from the coastal mountain range of southeastern Santa Catarina (including Ilha de Santa Catarina; Garcia, 1996) also refer to *E. henselii*. In northeastern Santa Catarina, there are several species involved, besides *E. henselii* and presumably *E. manezinho* (based on the recording from Guaramirím named rã-das-pedras, *Eleutherodactylus* sp., which is available at http://www.ra-bugio.org.br/index.html), at least one species which could be *E. guentheri*. All citations of *E. guentheri* from the Argentine province Misiones (Carrizo et al., 1989; Lavilla, 1992; Lavilla & Cei, 2001; Lavilla et al., 2002) and from southeastern Paraguay (Frost, 2002) presumably belong to *E. henselii*. Up to date, there are no records of *E. henselii* from the western regions (most parts of the *Araucaria* plateau) of Rio Grande do Sul and Santa

Catarina, whereas this species (or *E. guentheri*) occurs in western Paraná (Bernarde and Machado, 2001).

Natural history.—*Eleutherodactylus henselii* lives in mountain slopes with subtropical Atlantic rain forest and in *Araucaria* forests on the margin of the *Araucaria* plateau. It is a ground-living frog with diurnal and nocturnal activity. During the breeding season from October to January males call at night, rarely during the day, on the ground or from fallen trees and stones. Calling activity increases during warm periods with rainfall. Eggs are deposited terrestrially, below trunks or stones, as described for *E. guentheri* (Lutz, 1946). A big female of *E. henselii* (MCP 3183, SVL 37.7 mm, weigth 4.9 g), captured in activity on 4 December 1997 at 16 h, was dissected and contained 26 large (3 mm diameter) and 45 small (1 mm diameter) yellowish eggs.

DISCUSSION

Steindachner (1864) described *Hylodes guentheri* on the basis of a single specimen from Brazil without specific locality data. The holotype collected by Natterer (NHMW 16515) is a small specimen, presumably a small male or a subadult as stated by Heyer (1984), with following measurements (in mm): SVL 22.1, HL 9.2, HW 7.4; ED 2.7; TD 1.5; EN 3.2; IO 4.0; IN 1.8; TL 14.4; SL 15.5; FL 13.9. Bokermann (1966) indicated that the holotype probably came from Rio de Janeiro, which was accepted by Heyer (1984). Steindachner (1864) provided an accurate description of the holotype and later described and figured six additional specimens as varieties of *E. guentheri* (Steindachner 1867). One of us (AK) examined six individuals deposited in Vienna that have been collected by Natterer (NMW 4296, 13281), respectively the Novara Expedition from 1857-1859 (NMW 4292:1, 4292:2, 4294:1, 4294:2). These specimens represent the only material available from this locality and could be identical with the material described by Steindachner (1867). However, our examination revealed that only four specimens (NMW 4292:1, 4292:2, 4296 and 13281) are *E. guentheri*, whereas NMW 4294:1 and NMW 4294:2 have well developed foot webbing, not belonging to this species.

Hylodes henselii was described by Wilhelm C. H. Peters (1870), based on a specimen collected by Reinhold Hensel near Porto Alegre in the Brazilian state of Rio Grande do Sul. Hensel (1867) himself provided a short description of this frog but did not make taxonomic decisions because the specimen was dead and in poor condition when found. Boulenger (1882, 1886), who considered *Hylodes guentheri* Steindachner, 1864 a synonym of *Hylodes gollmeri* Peters, 1863, put *Hylodes henselii* in the synonymy of *Hylodes griseus* Hallowell, 1861 "1860". This arrangement was followed by Nieden (1923) and Miranda-Ribeiro (1926). Cochran (1955) revalidated *Hylodes guentheri* (as *Eleutherodactylus guentheri*) but she did not evaluate the status of *Hylodes henselii*. Therefore, this species continued to be a synonym of *Eleutherodactylus griseus* in the species list of Gorham (1966). Bokermann (1966), who listed *Hylodes henselii* (Peters, 1870) in his catalogue of the type localities of Brazilian anurans, confused it with *Physalaemus henselii* (Peters, 1872) and put both taxa together. However, *Physalaemus henselii*, originally described as *Paludicola henselii*, is a distinct species from Rio Grande do Sul which was described by Peters and dedicated to Hensel, but belongs to another distinct clade of *Eleutherodactylus henselii*.

When Savage (1974) allocated *Hylodes griseus* into the synonymy of *Eleutherodactylus fitzingeri*, a Middle American species, the south Brazilian *Hylodes henselii* remained as a valid but uncharacterized species. Lynch (1976), who revised the genus *Eleutherodactylus*, assigned *Hylodes henselii* tentatively to the *binotatus* group but stated that, "according to G. Peters (in litt.)", its holotype is lost and data are inadequate to definitely make a taxonomic decision. This erroneous communication of the then custos in the Berlin herpetological collection, Günther Peters, might be due to the fact that on the label of *Hylodes henselii* the original names had been crossed out and replaced by the name *Hylodes griseus*. Harding (1983:162) accepted *Eleutherodactylus henselii* as a full species but quoted eroneously Santa Catarina as type locality. In a revision of the *Eleutherodactylus guentheri* species cluster, Heyer (1984) put *Hylodes henselii* in the synonymy of *Hylodes guentheri* and designated NMW 16515 as a neotype, following the statement of Lynch (1976) that the holotype of *henselii* is lost. This arrangement was accepted by all subsequent authors (e.g. Frost, 1985, 2001; Lavilla, 1992), but our bioacoustic data revealed that the recovered holotype of *Hylodes henselii* (ZMB 6813, Bauer et al.; 1995) belongs to a cryptic species that must be removed from the synonymy of *E. guentheri*.

Carrizo et al. (1989) published the first record of *Eleutherodactylus guentheri* from Misiones, Argentina, based on three specimens (MACN 33083, MACN 33084, CENAI 4708). One of us (AK) examined this material. CENAI 4708 (in mm: SVL 36.4; HL 13.9; HW 13.1; ED 3.9; TD 2.3; IO 2.5; IN 2.7; TL 20.5; SL 23.9; FL 22.2) is an adult female fitting the description of *E. guentheri* (respectively *E. henselii*). Both MACN 33083 (SVL 21.4; HL 8.9; HW 8.2; ED 3.1; TD 1.5; IO 2.2; IN 1.9; TL 14.4; SL 14.8; FL 13.6) and MACN 33084 (SVL 24.3; HL 8.8; HW 8.8; ED 3.2; TD 1.6; IO 2.0; IN 2.0; TL 15.3; SL 16.6; FL 15.4) are adult males, varying in size, pattern and skin texture but seem to belong to the same species. Besides this material, Lavilla et al. (2002) reported two additional specimens from Misiones (Iguazú) assessing this species as insufficiently known in Argentina. The analysis of an acoustic recording from Misiones (Fig. 6B) assigned to *E. guentheri* showed that it actually belongs to *E. henselii*. However, further studies are needed to verify if all populations from Misiones are conspecific with *E. henselii* or if other species are involved.

The acoustic recording from Boracéia (Heyer et a., 1990), currently assigned to *E. guentheri,* is distinct from that of *E. guentheri* from near the type locality which suggests that it may represent another species. The overall similarity of the Boracéia call with that of *Eleutherodactylus manezinho* could either mean that both recordings belong to this species or that they represent two different but nearly related species of the *guentheri* cluster. The acoustic data contradict the morphological findings (Garcia, 1996; Castanho & Haddad, 2000) which suggest that *E. manezinho* is a member of the *lacteus* group of Lynch (1976). However, although not being a cryptic species, *E. manezinho* is morphologically so similar to *E. guentheri* that, in a large series of *Eleutherodactylus* from Ilha de Santa Catarina labeled as *E. guentheri* (BGSS 4381-94), we identified two specimens (BGSS 4382, 4389) belonging to *E. manezinho*. In his study on *Eleutherodactylus guentheri* and related species, Heyer (1984) described the monophyletic *guentheri* cluster, consisting of *E. epipedus, E. erythromerus, E. gualteri, E. guentheri, E. nasutus,* and *E. oeus*. *Eleutherodactylus izecksohni* was later included by Caramaschi and Kisteumacher (1988), and *Eleutherodactylus henselii* also belongs to this cluster. However, there are more calls referred to *E. guentheri* than available names and it seems that at least two or three other cryptic species are involved. These results confirm Heyer et al. (1996), who described a cryptic *Leptodactylus* species based solely on acoustic data and stated that sibling species pairs might be more common than currently acknowledged.

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APPENDIX 1

Specimens Examined

Eleutherodactylus henselii: Brazil: Rio Grande do Sul: near Porto Alegre (ZMB 6813, holotype); Canela (SMNS 9125); Maquiné (MCP 6192); São Francisco de Paula: Pró-Mata (MCP 1752, 2452-53, 2769, 3182-83, 3416, 3454, 3486, 3674, 3678-80, 3682-83, 3691-92, 3843, 3875, 4938, 6639-40, 6649-53, 6655, 6680, 6686, 7198, 7201; SMNS 9004-06, 9507); São Leopoldo (BGSS 5665-66); Torres (MCP 324-25, 327). Santa Catarina: Florianópolis: Ilha de Santa Catarina (BGSS 4381, 4383-88, 4390-94; MCP 1704-06); Rancho Queimado (MCP 1707); Teresópolis (= Queçaba, Águas Mornas) (NMW 4293:1-8, 15826:1-61, 19418;

USNM 137696-99). Argentina: Misiones: General Belgrano: Arroyo Monyolito (CENAI 4708); Dos de Mayo (MACN 33083-84).

Eleutherodactylus guentheri: Brazil: Santa Catarina: Blumenau (NMW 15826:62); Ilhota: Morro do Baú (MCP 1356, 1708-11); Pirabeiraba (USNM 235723-26). Paraná: Guaratuba (MCP 6300); Pinhão (MCP 2017, 2023, 2043). Minas Gerais: Belmiro Braga (MNRJ 27508); Monte Verde (MNRJ 30365-70). São Paulo: Juréia (USNM 303306-09); Ribeirão Branco (MNRJ 18706-07); Salesópolis: Boracéia (USNM 235630-33); São José do Barreiro (USNM 235699-702); Ilha de São Sebastião (BGSS 4348-80, MNRJ 23673-76). Rio de Janeiro (MNRJ 1835, 18706-07, 23673-76, 37508, 30365-67, 31665-66; NMW 4292:1-2, 4296, 13281); Angra dos Reis (USNM 096505-08); Rio dos Macacos (NMW 16515, holotype); Teresópolis (MNRJ 1835); Tijuca (USNM 097404-07).

Eleutherodactylus binotatus: Brazil: Rio Grande do Sul: Torres (MCP 329). Santa Catarina: Florianópolis: Ilha de Santa Catarina (MCP 6368-69, 6376, 6414, 6441). São Paulo: Ilha de São Sebastião (BGSS 4330-44).

Eleutherodactylus manezinho: Brazil: Santa Catarina: Florianópolis: Ilha de Santa Catarina (BGSS 4382, 4389; MCP 6364-67, 6413, 6425-29, 6442, 6445).

FIGURES

FIG. 1. Holotype (ZMB 6813, female) and recently captured female (SMNS 9125) of *Eleutherodactylus henselii*.

FIG. 2. *Eleutherodactylus henselii*, lateral views of head of (A) ZMB 6813, (B) SMNS 9125; ventral views of right hand (C) and right foot (D) of SMNS 9125.

FIG. 3. *Eleutherodactylus henselii* in life from Rio Grande do Sul, showing variation in dorsal pattern; (A, B) adult males, (C, D) adult females.

FIG. 4. *Eleutherodactylus guentheri*, NMW 16515 (holotype), dorsal view (A), lateral view of head (B).

FIG. 5. Audiospectrograms (above) and oscillograms (below) of two call types of *Eleutherodactylus henselii* from Pró-Mata, Rio Grande do Sul; prolonged advertisement call (A), short aggressive call (B).

FIG. 6. Oscillograms of the advertisement call of *Eleutherodactylus henselii* from Guaramirím, Santa Catarina (A), Misiones, Argentina (B) and Pró-Mata, Rio Grande do Sul (C) in comparison with the advertisement call of *Eleutherodactylus guentheri* from Serra da Bocaina, São Paulo (D).

FIG. 7. Audiospectrograms (above) and oscillograms (below) of the advertisement call of *Eleutherodactylus guentheri* from Serra da Bocaina, São Paulo (A), Morro do Baú, Santa Catarina (B), and Guaramirím, Santa Catarina (C).

FIG. 8. Audiospectrograms (above) and oscillograms (below) of short call types of *Eleutherodactylus guentheri* from Pirabeiraba, Santa Catarina (A), Guaramirim, Santa Catarina (B), Pró-Mata, Rio Grande do Sul (C), Boracéia, São Paulo (D), and of the advertisement call of *Eleutherodactylus manezinho* from Ilha de Santa Catarina (E).

TABLE 1. Measurements (in mm) of adult males and females of *Eleutherodactylus henselii* from Rio Grande do Sul and southern Santa Catarina and *Eleutherodactylus guentheri* from Rio de Janeiro (specimens listed in Appendix 1).

Eleutherodactylus henselii	Males (n = 45)			Females (n = 41)		
	Range	Mean	SD	Range	Mean	SD
Snout-vent length	21.0-27.5	24.3	1.54	28.4-38.4	33.4	2.39
Head length	9.0-11.8	10.3	0.68	11.6-16.0	13.9	1.08
Head width	7.0-9.5	8.4	0.58	9.8-14.1	11.8	0.96
Tympanum diameter	1.1-1.8	1.4	0.22	1.6-2.9	2.0	0.25
Eye diameter	1.8-3.5	2.6	0.41	2.3-4.4	3.3	0.45
Interorbital distance	3.7-5.4	4.6	0.41	4.9-7.7	5.9	0.57
Internarial distance	1.8-2.8	2.3	0.25	2.5-3.5	3.0	0.26
Eye-nostril distance	2.2-3.9	3.2	0.31	3.6-5.3	4.3	0.35
Thigh length	12.6-17.7	14.9	1.09	18.1-24.3	20.7	1.53
Shank length	14.4-18.5	16.5	0.96	20.3-26.3	23.1	1.42
Foot length	12.2-16.5	14.6	0.86	17.2-23.3	20.3	1.43
Eleutherodactylus guentheri	Males (n = 10)			Females (n = 8)		
	Range	Mean	SD	Range	Mean	SD
Snout-vent length	20.2-28.3	24.1	2.60	31.0-40.5	34.7	3.01
Head length	7.9-12.1	10.1	1.33	12.2-16.3	14.2	1.14
Head width	6.6-9.7	8.3	1.01	11.3-14.5	12.4	1.11
Tympanum diameter	1.3-1.9	1.5	0.18	1.7-2.4	2.1	0.23
Eye diameter	1.7-2.7	2.2	0.40	2.8-4.0	3.4	0.47
Interorbital distance	3.5-5.3	4.3	0.62	5.4-7.7	6.3	0.71
Internarial distance	1.8-2.7	2.2	0.26	2.6-4.2	3.2	0.50
Eye-nostril distance	2.7-3.8	3.2	0.35	3.8-5.4	4.6	0.56
Thigh length	12.3-17.5	14.9	1.53	19.7-23.3	21.2	1.24
Shank length	13.5-20.2	16.4	1.90	21.1-26.1	23.3	1.69
Foot length	12.6-17.6	14.7	1.63	18.4-22.6	20.9	1.54

TABLE 2. Call parameters in *Eleutherodactylus henselii* from Rio Grande do Sul (RS), Santa Catarina (SC) and Argentina, *Eleutherodactylus manezinho* and different populations of *Eleutherodactylus guentheri* from São Paulo (SP) and SC. Temporal data are given as mean, followed by range in parentheses.

Identification	Air temperature (C)	Number of calls (specimens)	Call duration (sec)	Dominant frequency range (Hz)	Harmonic (Hz)	Note duration (msec)	Note interval (msec)	Notes per call	Note repetition rate (notes/sec)
E. henselii									
São Francisco de Paula, RS									
Advertisement call (long)	19	10 (2)	17.5 (12.4-23.0)	2200-3100	4000-4600	3.5-5.5	110 (90-196)	96-170	5-9
Encounter call (short)	19	10 (2)	0.50 (0.44-0.53)	2300-3100	4000-4900	4.0-5.0	82 (77-96)	5-6	10-12
Ilha Santa Catarina (long)	20.5	2 (1)	11.4 (9.5-13.3)	2300-2900	3800-4400	3.5-5.0	79 (70-86)	86-104	10-11
Guaramirím, SC (long)	—	1 (1)	14.4	2300-2800	3700-5200	5.0-6.0	102 (51-186)	126	8-10
Misiones, Argentina (long)	—	1 (1)	17.9	2100-2700	3700-4100	4.0-6.0	124 (99-227)	118	5-8
E. guentheri									
Bocaina, SP (long)	19-20	3 (1)	1.2 (0.8-1.8)	1800-2500	3400-3800	4-5	42 (40-44)	11-36	19-20
Morro do Baú, SC (long)	17.5	3 (1)	2.4 (2.1-2.7)	2200-3000	3900-4400	4-5	83 (78-91)	22-28	11-12
Guaramirím, SC (long)	—	1 (1)	1.0	2100-3100	3900-4600	3.5-5	69 (62-74)	14	14
Guaramirím, SC (short)	—	4 (1)	0.24 (0.20-0.28)	2600-3200	4100-4800	4-5	69 (44-76)	3-4	15-16
Pirabeiraba, SC (long)	—	3 (1)	1.6 (1.1-1.8)	1900-2900	3600-4100	4-6	61 (57-67)	19-28	16-17
Pirabeiraba, SC (short)	—	3 (1)	0.43 (0.24-0.59)	1800-2800	3500-4300	3-5	47 (40-61)	5-10	18-19
Boracéia, SP (short)	19.2	6 (2)	0.54 (0.44-0.64)	2500-3200	—	9-24	61 (48-76)	7-10	16-17
E. manezinho									
Ilha de Santa Catarina	20.5-23	10 (2)	0.56	2200-3200	4400-4900	12-25	76 (65-96)	7	11-14

Fig. 1

Fig. 2 A, B

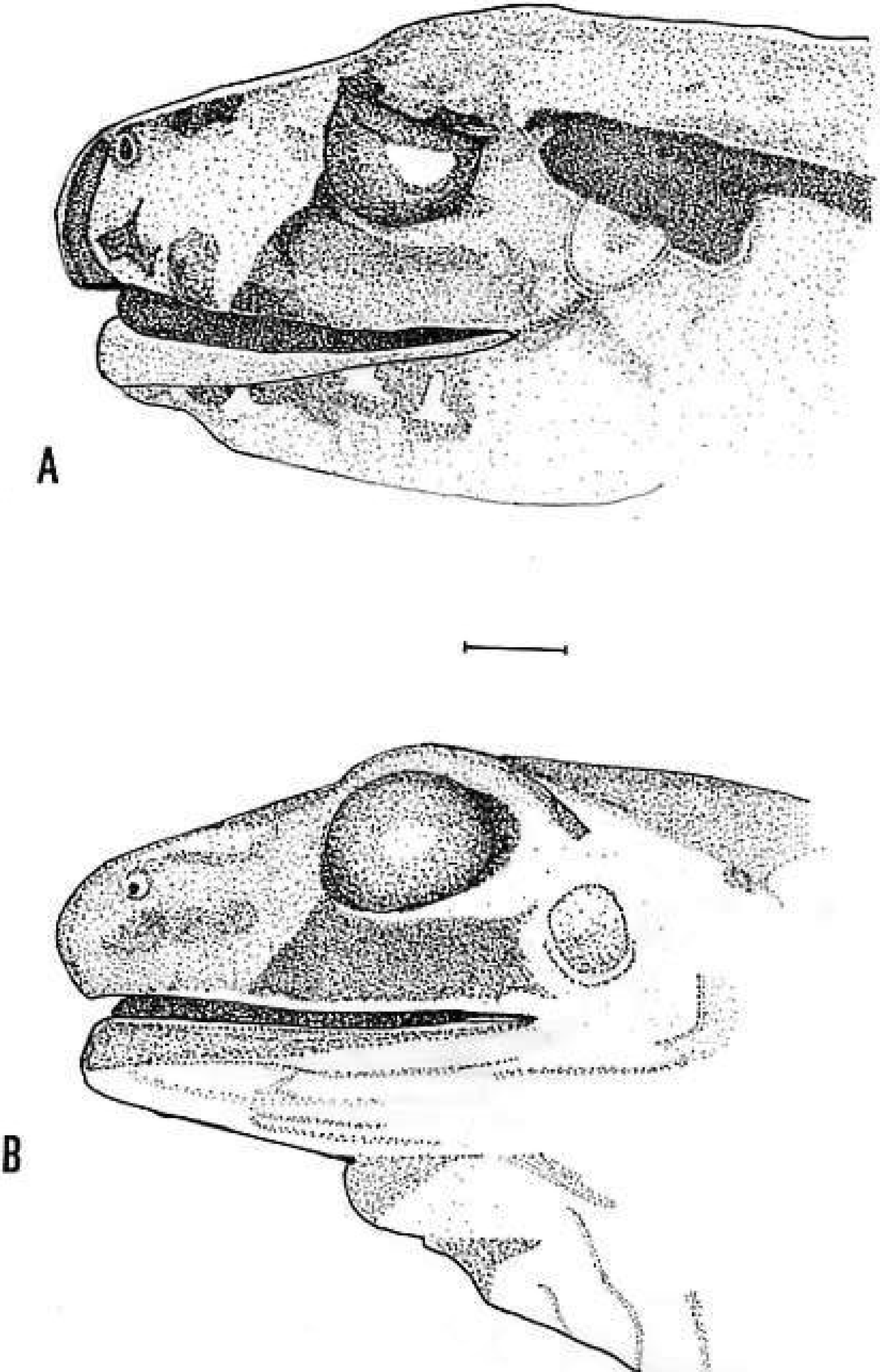


Fig. 2 C

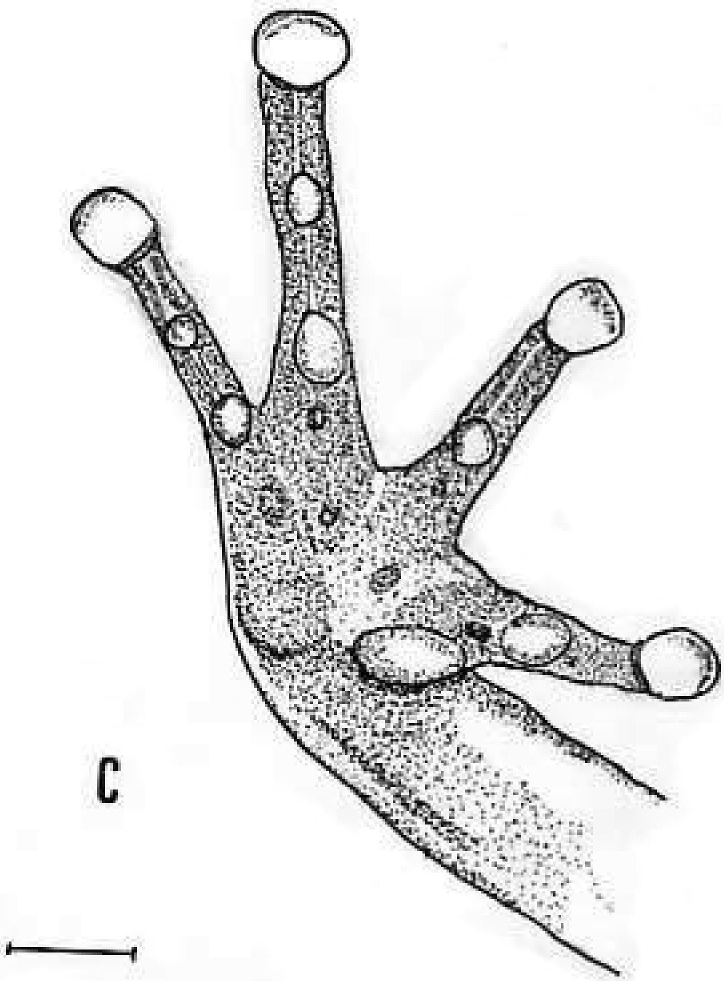


Fig. 3 A, B, C, D

Fig. 4 A, B

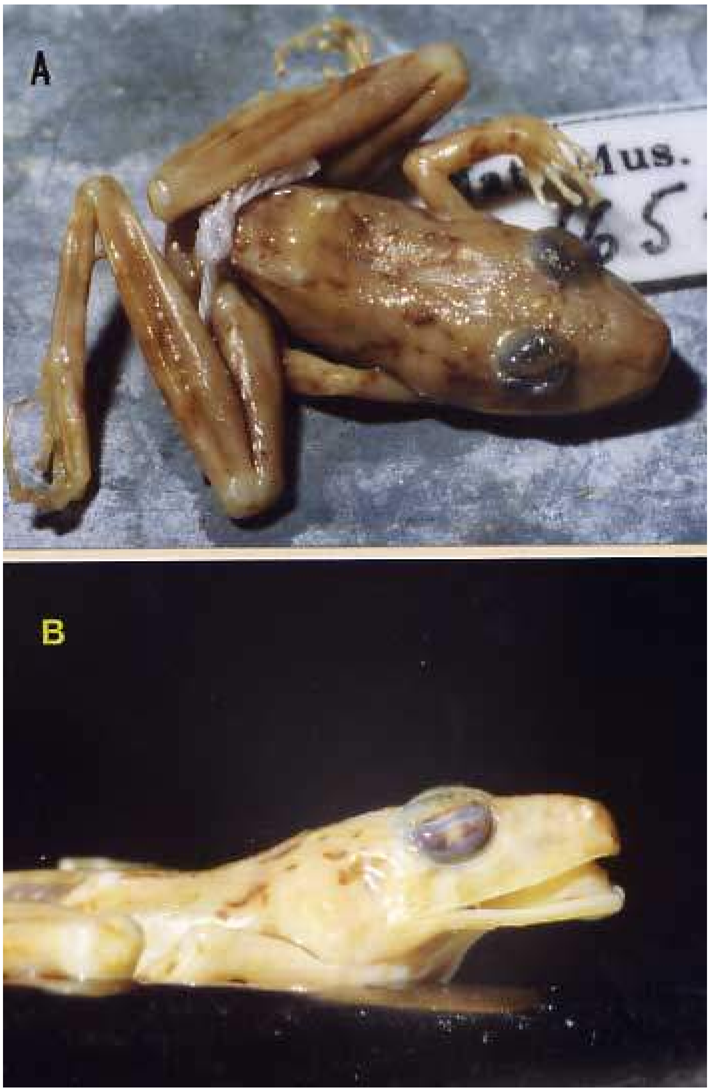


Fig. 5

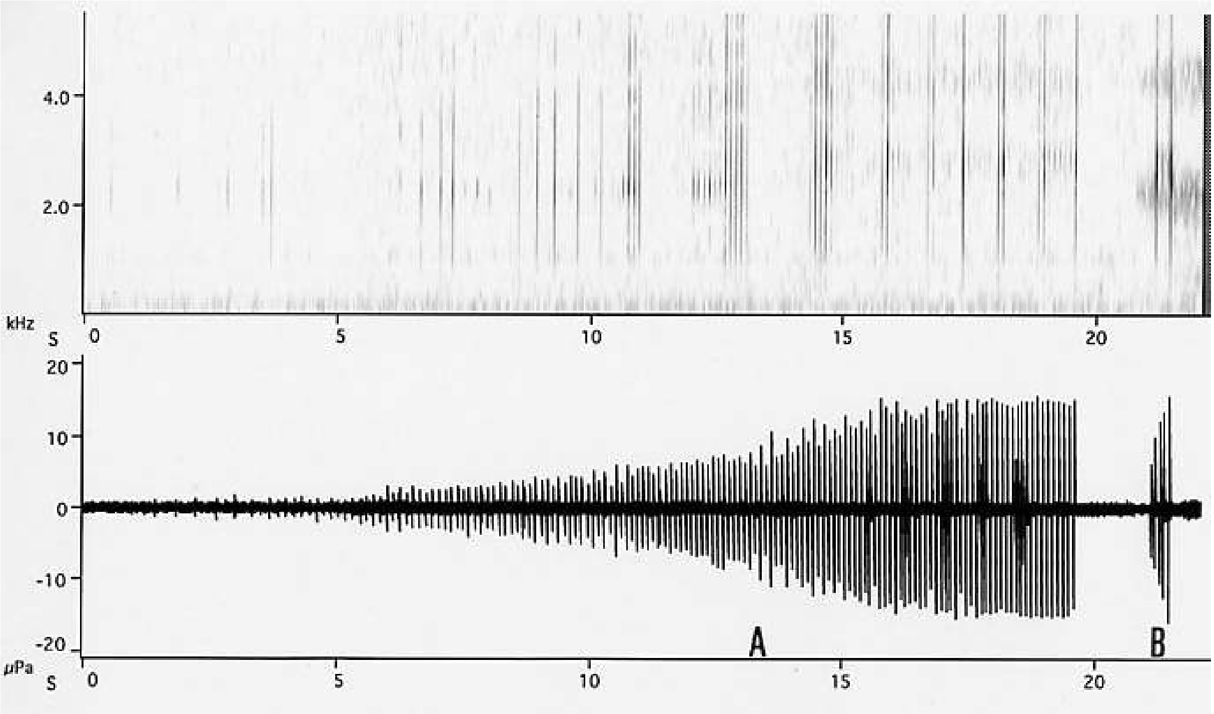


Fig. 6

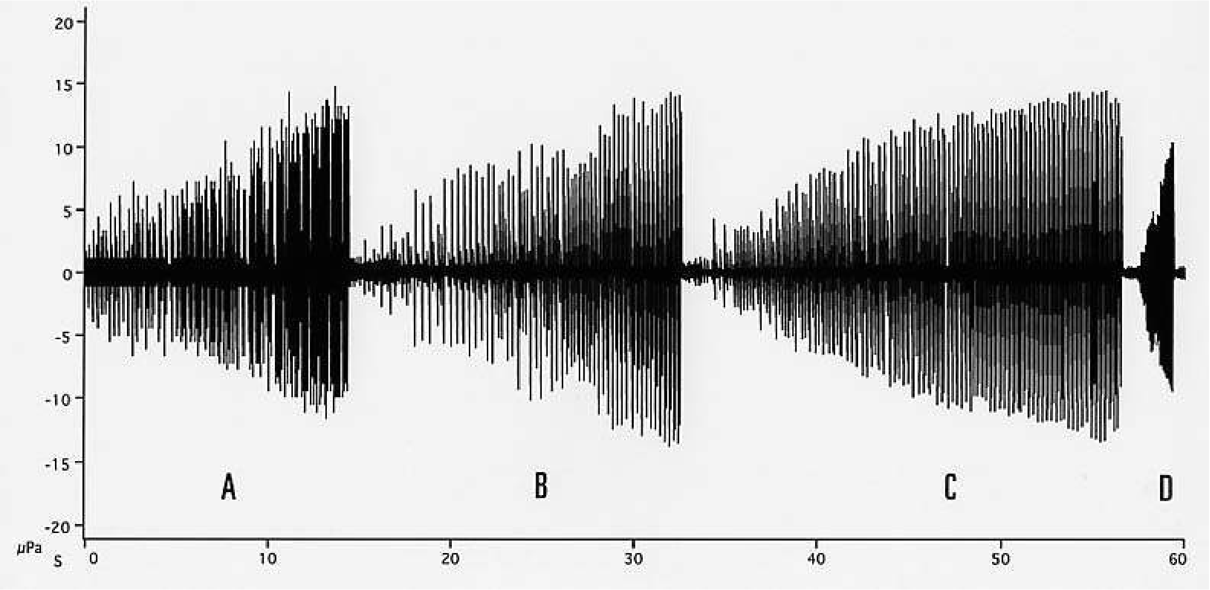


Fig. 7

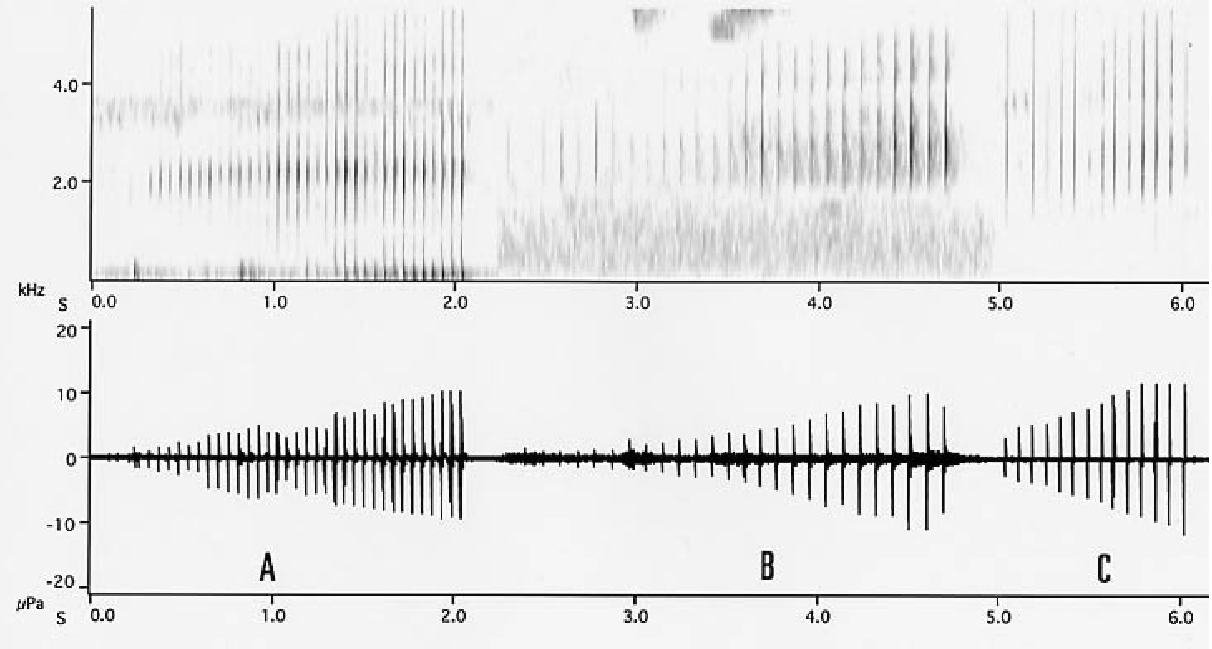
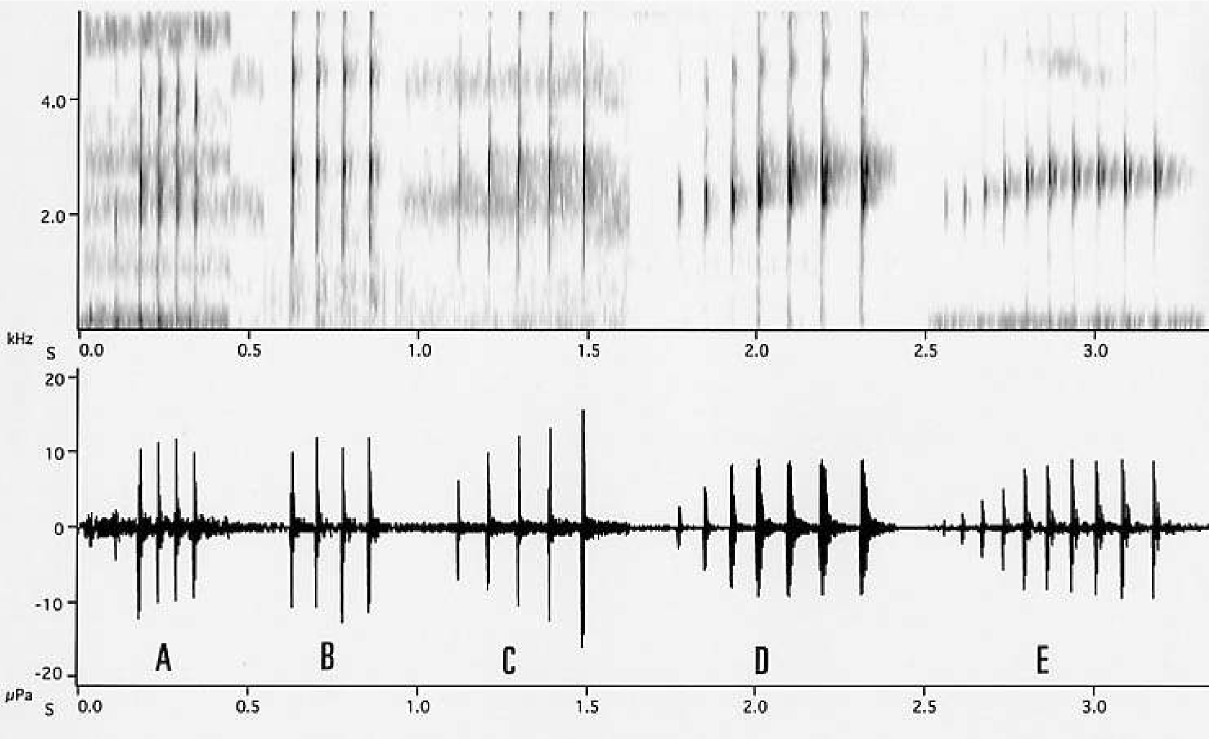


Fig. 8



5.5. Both, Camila[1]; Kwet, Axel[2] & **Solé, Mirco[3]**

The tadpole of *Physalaemus lisei* Braun & Braun, 1977 from southern Brazil (Anura: Leptodactylidae)

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Alytes (2004, eingereicht)

Die Gattung *Physalaemus* umfasst 41 beschriebene Arten. Davon kommen acht im Bundesstaat Rio Grande do Sul vor. Die Kaulquappen dreier dieser Arten waren noch nicht bekannt. Wir sammelten im Waldschutzgebiet Pró-Mata ein adultes Pärchen der Art *Physalaemus lisei* und beobachteten den Bau des Schaumnests. Die Kaulquappen wurden gleich nach dem Schlupfvorgang in 100 x 100 x 40 cm messende Wasserlöcher überführt und nach 9, 33 und 45 Tagen vermessen. Für die Analyse der morphometrischen Daten wurden 27 Kaulquappen benutzt. Die Beschreibung der externen und internen Morphologie der Larve wurde an einer dieser Kaulquappen im Gosner-Stadium 37 durchgeführt. Die ermittelte Zahnformel lautet 2(2)/3(1). Die Untersuchung der internen Strukturen anhand von rasterelektronenmikroskopischer Aufnahmen zeigten einen gut ausgebildeten Buccopharynx mit einem U-förmigen Mundhöhlendach mit vier fingerförmigen Papillen und einem dreieckigen Boden mit sechs mehrmals verzweigten Infralabialpapillen und fünf Lingualpapillen. Die

Kaulquappen der Gattung *Physalaemus* sehen sich sehr ähnlich. Für viele Arten kann die Morphologie des Mundfelds nicht zur sicheren taxonomischen Bestimmung herangezogen werden.

The tadpole of *Physalaemus lisei* Braun & Braun, 1977 from southern Brazil (Anura: Leptodactylidae)

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Contents: 8 pages, 2 tables and 3 figures.

ABSTRACT

The tadpole of *Physalaemus lisei* is described for the first time. Data on the external and internal morphology, together with notes on the life history are presented. A comparison is made including all available information on tadpoles of the *Physalaemus* species from Rio Grande do Sul State.

INTRODUCTION

The genus *Physalaemus* comprises 41 described species, which are distributed in Central and South America, ranging from Mexico to Uruguay and adjacent Argentina (FROST, 2002). Eight species of *Physalaemus* are currently known from Rio Grande do Sul, southern Brazil: *P. biligonigerus*, *P. cuvieri*, *P. gracilis*, *P. henselii*, *P. lisei, P. nanus, P.*

riograndensis, and several undescribed taxa (KWET, 2001). The external morphology of the tadpoles of five of these species has been previously described, *P. biligonigerus* (FERNÁNDEZ & FERNÁNDEZ, 1921), *P. cuvieri* (BOKERMANN, 1962; HEYER et. al, 1990), *P. gracilis* (LANGONE, 1989), *P. henselii* (BARRIO, 1953; BARRIO, 1964), and *P. riograndensis* (KEHR, 2004), whereas the larvae of *P. lisei* and *P. nanus* remain unknown.

Physalaemus lisei occurs in the mountain region of northwestern Rio Grande do Sul (BRAUN & BRAUN, 1977). The species usually inhabits damp woodland and is frequently found in secondary forests or transition zones from woodland to grassland (KWET & DI-BERNARDO, 1999). We provide additional data on the life history of *P. lisei* and describe for the first time the external morphology of the tadpole. This description is compared with those of other congeneric tadpoles from Rio Grande do Sul.

MATERIALS AND METHODS

The material originated from the Centro de Pesquisa e Conservação da Natureza Pró-Mata, municipality of São Francisco de Paula, Serra Geral region of Rio Grande do Sul, Brazil, at 29°27’ - 29°35 S and 50°08’ – 50°15’ W (KWET, 2001) . Amplectant pairs of *Physalaemus lisei* were collected at temporary ponds and maintained in captivity until spawning. Foam nests were transferred to an artificial pond measuring 100 x 100 x 40 cm excavated on a field near the collection site. Previously we raised tadpoles in aquaria , but these tadpoles often showed reduced growth rates and malformations of the oral apparatus. The artificial pond was filled with water two days before the eggs were transferred to avoid eventual predators. The larvae fed on algae and detritus naturally occurring in the pond. We did not provide additional food.

Tadpoles were collected on days 9, 33 and 45 after hatching. Larvae were conserved in alcohol 70% and deposited in the collection of the MCT (Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul) (App. 1). We analysed 27 specimens (Tab. 1). Measurements were taken to the nearest 0.01mm with a stereomicroscope, following the terminology of ALTIG & MCDIARMID (1999). The labial tooth row formula (LTRF) and developmental stages follow ALTIG (1970) and GOSNER (1960). The internal oral anatomy was studied under a scanning electron microscope. The terminology follows WASSERSUG (1976).

RESULTS

PHYSALAEMUS LISEI BRAUN & BRAUN, 1977

Description of the external morpholoy - Body oval in dorsal view, depressed; body approximately 40.1% (± 2.9 according to the stage) of total length (Fig. 1). Snout rounded in dorsal and lateral view. Nostrils round, directed dorsolaterally; closer to eyes than to snout; internarial distance approximately 48% (± 5.3) of interorbital distance. Eyes dorsal, directed laterally. Spiracle sinistral, located anterior to midbody; lateral wall not free, directed posteriorly. Anal tube dextral, directed posteriorly.

Tail higher than body, about 59.8% (± 2.9) of total length. Dorsal fin convex, ventral fin almost straight, origin of dorsal fin slightly precedes the body-tail juncture. Fins gently tapering to acuminate tip.

Oral disc anteroventral, width 35.4% (± 6.1) of body width (Fig. 2). Lower jaw sheath and upper jaw sheath queratinized. Upper jaw sheath arch-shaped, lower jaw sheath V-shaped, both wider than high and finely serrate. A single row of marginal papillae surrounds the oral disk, an extensive rostral gap is present, no mental gap. Intramarginal papillae are absent. Labial teeth small, closely spaced. Tooth row formula 2(2)/3(1).

In preserved specimens, gut visible by transparency. Some specimens have a brownish coloration visible on dorsum and on the tail muscle. The area surrounding the eyes is overall lighter. Tail fins transparent with irregular brownish marks.

Internal oral structures - Buccal roof (Fig. 3A) elongated with semicircular prenarial and posnarial arena. Posnarial arena with two long lateral ridge papillae. Internal nares oblique in orientation. Narial valve projection ornamented with irregular pustulations. Medial ridge slightly inclined towards the rostrum, overall trapezoidal in shape, its width approximately 50% of the width of the postnarail arena, and having a postulated edge. Buccal roof arena U-shaped defined by four long and finger-like papillae on each side, buccal roof arena with scattered pustulations. Glandular zone with limits in semi-circular form, elevate on lateral parts.

Buccal floor (Fig. 3B) triangular, shorter than the buccal roof. Presence of six multiple-branching infralabial papillae postulated, four near the lower beak and two positioned posterior. Five lingual papillae localized between the two last infralabial papillae, placed in the medial width of the tongue. Four are finger-like shaped, two on each side and closely spaced, laterally localized and a medial bifurcate papillae, larger with ramifications. Ventral velum clearly visible with flaps in the medial part. Pustulations present on the flaps.

Notes on life history - We found 12 foam nests, each of which 3-4 cm in diameter, the minimum number of eggs counted were 397 and the maximum 779, with an average of 539 eggs.

Two large, collective foam nests were found at a paddock in the study site. One nest containing 2004 eggs was observed on 20/XI/98 in a small pond measuring 0.75 x 0.75 m, whereas the other containing 1355 eggs was detected on 10/I/1999 in a flooded area.

Larvae of *Physalaemus lisei* were often observed in temporary ponds between or under stones and fallen leaves, scraping algae fixed on stones or particles deposited on the bottom. During feeding tadpoles move slowly and preferentially use the bottom of shallow waters.

DISCUSSION

All tadpoles of *Physalaemus* from RS are similar in external morphology. In all species, the body is ovoid in dorsal view and wider than its height; the vent tube is dextral and the spiracle is sinistral. Eyes are dorsolateral; the oral disc is anteroventral and the overall coloration is brownish. The measurements of larval *P. lisei* varied considerably between different stages pointing out that morphometric data might not be suitable characteristics for differentiation between tadpoles of the different species of *Physalaemus*.

The oral morphology is variable and allows the differentiation among tadpoles of some species. Tadpoles of the *P. cuvieri* species group from Argentina can be distinguished based on their oral disc morphology (KEHR et al., 2004). The same analysis allows the distinction of the tadpoles of *P. cuvieri*, *P. henselii*, *P. lisei* and *P. riograndensis* (*P. cuvieri* species group) and *P. biligonigerus* and *P. gracilis* (*P. biligonigerus* species group) present in RS state. *Physalaemus biligonigerus and P. riograndensis* can easily be distinguished from other species by presenting only two lower tooth rows and from each other by their different tooth row formula*:* *P. biligonigerus* 2(2)/2 (FERNÁNDEZ & FERNÁNDEZ, 1921) and *P. riograndensis* 2(2)/2(1) (LANGONE, 1989) (Tab. 2).

In some species of *Physalaemus* the oral morphology was described using tadpoles from different populations. *Physalaemus henselli* was first described by BARRIO (1953) with the tooth row formula 2(2)/3(1), whereas the same species was later described as having the formula 2/3(1) (BARRIO, 1964). Also, BOKERMANN (1962) and CEI (1980) recorded for *P. cuvieri* the tooth row formula 2/3(1), whilst HEYER et al. (1990) suggested the tooth row formula 2(2)/3(1). For *Physalaemus henselii* and *P. cuvieri* the tooth row formula, 2/3(1) was also reported (BARRIO, 1964; BOKERMANN, 1962). Both can be differentiated by the number of marginal papillae. *Physalaemus cuvieri* has a single row of marginal papillae and *P. henselii* has two rows at the supra and infraangular region. According to BARRIO (1953) and HEYER et al. (1990), both *P. henselii* and *P. cuvieri* show the tooth row formula 2(2)/3(1) which is the same as in *P. gracilis* and *P. lisei*. In this case, the marginal papillae might be used to differentiate between them. *Physalaemus cuvieri* and *P. lisei* have a single row of marginal papillae, whilst *P. lisei* and *P. gracilis* present a double row. *Physalaeums cuvieri* can be distinguished from *P. lisei* by having a rostral and a mental gap, whilst *P. lisei* has only a rostral gap. *P. henselii* and *P. gracilis* can also be differentiated by the gaps disposition: the first has mental and rostral gaps, whilst the second has only a rostral gap.

In several species of *Physalaemus* oral morphology cannot be used for unambiguous differentiation of tadpoles, e.g., in *P. bokermanni* (CARDOSO & HADDAD; 1985) and *P. maculiventris* (BOKERMANN, 1963) the same tooth row formula is represented, with number and position of marginal papillae as *P. lisei*.

Physalaemus biligonigerus seems to possess the largest foam nests within all species of *Physalaemus* in RS, measuring 10-15 cm in diameter (FERNANDEZ & FERNANDEZ, 1921). *P. cuvieri* has mid-sized foam nests of 7-9 cm (Cei, 1980), respectively 5-6 cm (KWET & DI-BERNARDO, 1999) in diameter, containing 300-400 eggs. *Physalaemus lisei* and *P. henselii* have small, 3-4 cm sized foam nests. In foam nests of *P. henselii,* Barrio (1953) found 200-250 eggs and CEI (1980) 250-300 eggs. In the present study, we observed egg numbers in *P. lisei* varying between 300 and 700 eggs. There are no data available on *P. gracilis*. Additional data are required to distinguish foam nests of the different species of *Physalaemus*.

RESUMO

La larva de *Physalaemus lisei* es descrita por primera vez. Aportamos datos sobre la morfología interna y externa junto con notas sobre la história natural. Comparamos toda la información disponible sobre larvas de *Physalaemus* del estado de Rio Grande do Sul.

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APPENDIX 1

Specimens examined. *Physalaemus lisei*. – MCP 3889, nine tadpoles collected on 4 January 1999. MCP 3890, four tadpoles collected on 4 January 1999. MCP 3891, 10 tadpoles collected on 23 December 1998. MCP 3892, 7 tadpoles collected on 23 December 1998. MCP 3895, 10 tadpoles collected on 29 November 1998. MCP 4953, 7 tadpoles collected on 22 January 2001. All tadpoles were collected at the CPCN "Pró-Mata", at 29°27' - 29°35 S and 50°08' – 50°15'W, by M. Solé.

TABLE 1. Measurements (mean; in millimeters) of tadpoles of *Physalaemus lisei*.

Stage	25 N=10	27 N=2	28 N=7	32 N=2	34 N=1	36 N=1	37 N=3	40 N= 1
Total length	10.9	13.8	16.8	17.5	17.7	19.1	23.7	25.2
Body length	4.4	5.8	6.7	7.4	7.1	7.8	9.8	9.5
Body width	3.2	4.2	4.9	5.5	6.0	5.8	7.3	6.0
Body height	2.6	3.6	3.5	4.5	4.5	4.9	5.5	4.5
Tail length	6.5	8.1	10.2	10.1	10.6	11.2	13.9	15.7
Eye diameter	0.4	0.6	0.7	0.8	0.8	0.9	1.1	1.2
Oral disc width	1.2	1.3	1.7	1.7	1.8	2.0	2.2	2.2
Inter orbital distance	1.4	1.5	1.8	2.2	2.3	2.3	3.0	2.9
Inter narial distance	0.7	0.7	0.9	1.0	1.1	1.2	1.4	1.4
Eye-nostril distance	0.6	0.8	0.9	1.0	1.2	1.2	1.7	1.7
Nostril-snout distance	1.1	1.2	1.4	1.2	1.4	1.4	2.0	2.2

Table 2. Comparison of oral morphological features of *Physalaemus* species tadpoles from RS (South Brazil).

	Tooth row formula	Marginal papillae row	Rostral gap	Mental gap
P. biligonigerus Fernández y Fernández (1921)	2(2)/2	Single	Presente	Absent
P. cuvieri Bokermann (1962)	2/3(1)	Single	Present	Present
P. cuvieri Heyer et al (1990)	2(2)/3(1)	Single	Present	Present
P.gracilis Langone (1989)	2(2)/3(1)	Double	Present	Absent
P. henselii Barrio (1953)	2(2)/3(1)	Mental single, supra- and infraangular double	Present	Present
P. henselii Barrio (1964)	2/3(1)	Mental single, supra- and infraangular double	Present	Present
P. lisei Described in this paper	2(2)/3(1)	Single	Present	Absent
P. riograndensis Kehr et al (2004)	2(2)/3(1)	Single	Present	Absent

Fig. 1 — Tadpole of *Physalaemus lisei* (stage 37): lateral view (scale line: 1 cm)

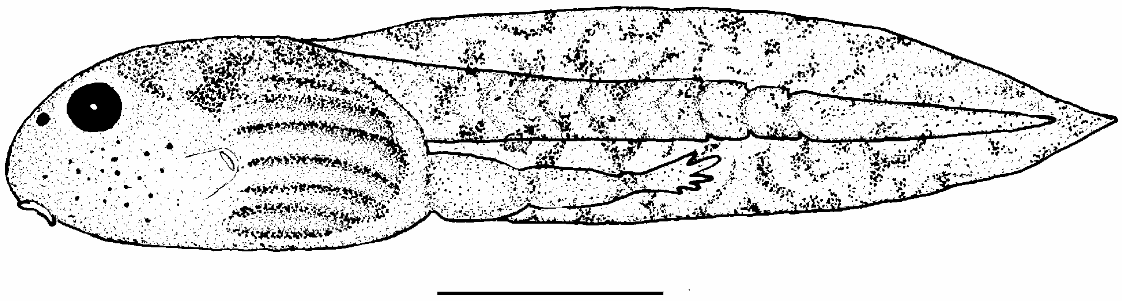


Fig. 2 — Tadpole of *Physalaemus lisei*: oral disc (scale line: 2 mm)

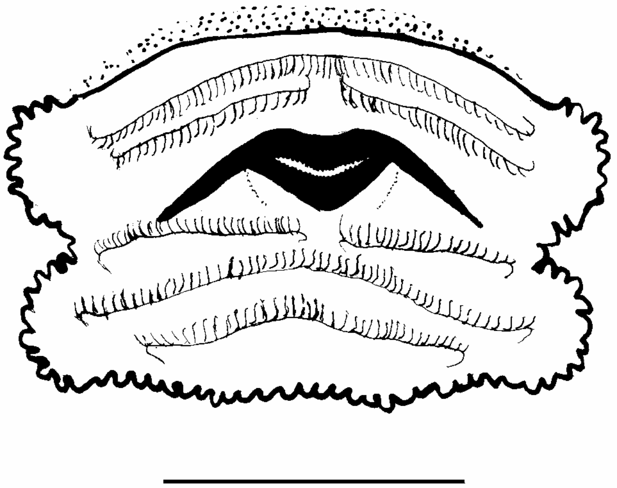
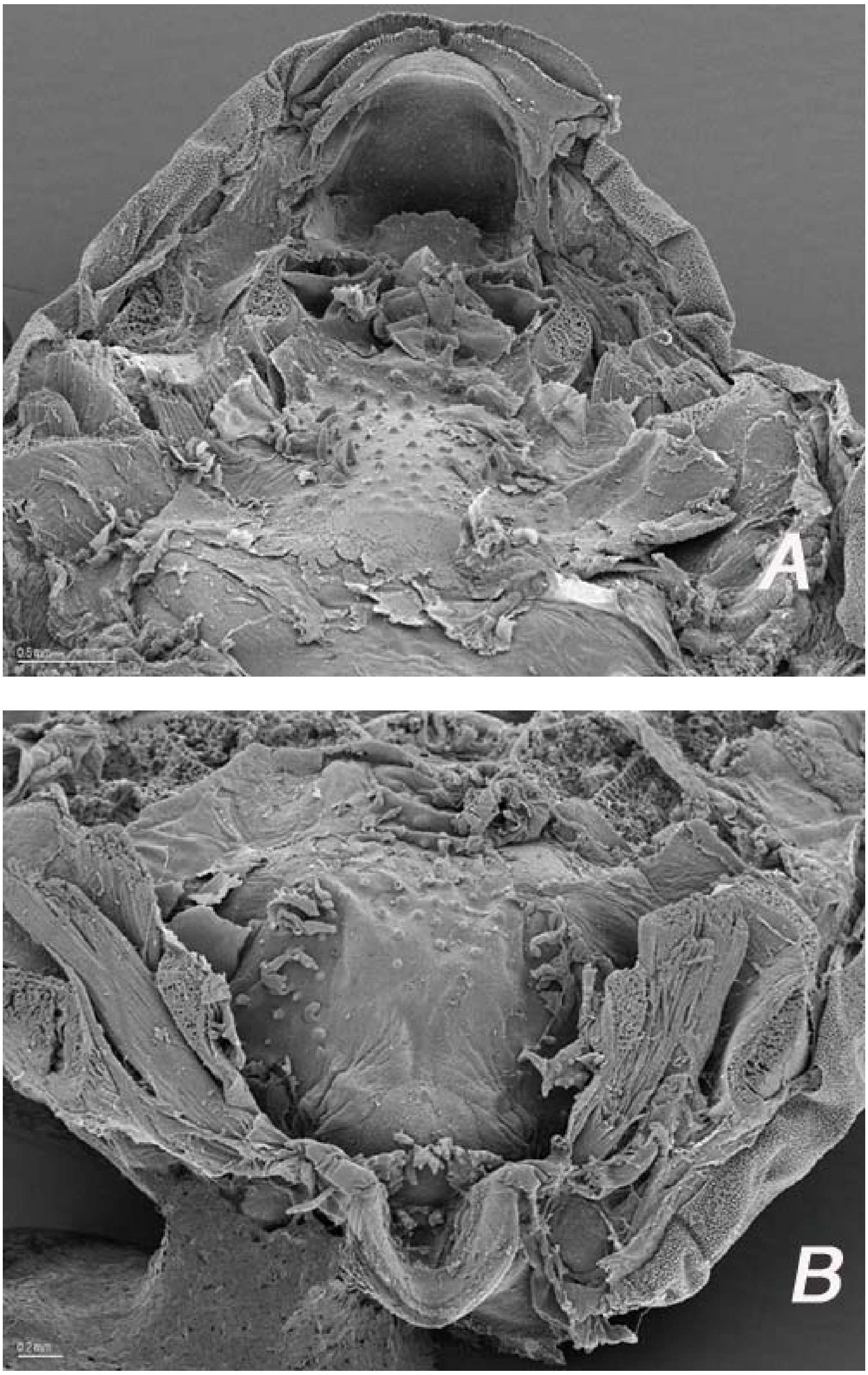


Fig. 3. — Section of the mouth of the tadpole of *Physalaemus lisei* at 37 Stage according to Gosner (1960). In A, the buccal roof of the oral cavity (Bar = 0,5mm) and in B, the buccal floor (Bar = 0,2 mm).



5.6. Both, Camila[1,2]; Kwet, Axel[2,3] & **Solé, Mirco[2]**

The tadpole of *Hyla leptolineata* (Braun & Braun, 1977), a species in the *Hyla polytaenia* group (Anura, Hylidae).

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Die *Hyla polytaenia*-Gruppe umfasst sieben Arten. Das Verbreitungsgebiet einer dieser Arten (*Hyla leptolineata*) reicht bis in den Norden des Bundesstaats Rio Grande do Sul. Im Waldschutzgebiet Pró-Mata wurden 17 Kaulquappen gesammelt und die äußere und innere Morphologie untersucht. Der graubraune ovale Körper macht 32% der Gesamtlänge aus. Die Schwanzflossen sind konvex und entspringen vor der Basis des Schwanzmuskels. Das Mundfeld weist die Zahnformel 2(2)/3(1) auf. Die kleinen Labialzähnchen liegen eng beieinander. Das Mundhöhlendach besteht aus gut erkennbaren pre- und postnarialen Feldern. Seitlich befindet sich jeweils eine wohl ausgebildete Papillenreihen. Der Mundhöhlenboden zeichnet sich durch eine dreieckige Form aus. Lateral befinden sich zwei verzweigte Papillen. Zwei symmetrische Lingualpapillen sind in der Mitte der Zunge angeordnet. Die Kaulquappen aller Arten der *Hyla polytaenia*-Gruppe können anhand ihrer Mundfelder unterschieden werden. *Hyla* *leptolineata* weist zwar die selbe Zahnformel wie *H. cipoensis* auf, die Anordnung der Marginalpapillen ist jedoch unterschiedlich gestaltet: Während *H. cipoensis* auf der Unterlippe zwei Papillenreihen hat, weist *H. leptolineata* nur eine Reihe auf.

The Tadpole of *Hyla leptolineata* (Braun & Braun, 1977), a Species in the *Hyla polytaenia* Species Group (Anura, Hylidae)

CAMILA BOTH,[1,2] AXEL KWET[2,3] AND MIRCO SOLÉ[2,4]

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ABSTRACT – **The larval morphology of *Hyla leptolineata* was studied. The body of the tadpole is ovoid in dorsal view, more wide than deep. The snout is rounded with reniform nostrils located dorsally. The spiracle is sinistral with lateral wall attached to the body, the anal tube is dextral. The tail fin is convex, ending in an acuminate tip. The oral disc lies ventrally. The labial tooth row formula is 2(2)/3(1). The larvae present moderately developed beaks with serrated jaw sheats. These external oral features are compared with those of the known tadpoles in the *Hyla polytaenia* species group. The oral cavity was studied using an electron microscope. Life history aspects are commented on.**

Keywords: Anura, Hylidae, Tadpole, *Hyla leptolineata.*

INTRODUCTION

The *Hyla polytaenia* group was defined by Cruz and Caramaschi (1998), based on external morphological features and coloration patterns. This group, which is distributed in the central west, southeast and south of Brazil, currently comprises seven species: *H. buriti*, *H. cipoensis*, *H. goiana*, *H. leptolineata*, *H. phaeopleura*, *H. polytaenia* and *H. stenocephala* (Cruz and Caramaschi, 1998; Caramaschi and Cruz, 1999, 2000; Frost, 2004). Cruz and Caramaschi (1998) and Eterovick et al. (2002) analysed the taxonomic position of the *polytaenia* group and discussed its proximity to the *Hyla pulchella* species group. Recent molecular studies suggest that the *Hyla polytaenia* clade is nested within the *Hyla pulchella* group (Faivovich et al., 2004).

Hyla leptolineata has been described from southern Brazil by Braun & Braun (1977). Kwet (2001) studied reproductive aspects and vocalization patterns of this species, but its tadpole remains unknown. Within the *Hyla polytaenia* group, external tadpole morphology has been described for *Hyla polytaenia* (Heyer, 1990), *Hyla goiana* and *Hyla cipoensis* (Eterovick et al., 2002). In our paper, we describe the larval morphology of *Hyla leptolineata* and compare it with morphological characteristics of these previously described tadpoles. The morphology of the internal oral cavity of *Hyla leptolineata* is also described.

MATERIAL AND METHODS

Tadpoles were collected at the Centro de Pesquisa e Conservação da Natureza Pró-Mata, municipality of São Francisco de Paula, Serra Geral region of

Rio Grande do Sul, Brazil (29°27’-29°35 S and 50°08’-50°15’ W) between 1995 and 1997 by AK. The larvae were stored in 70% alcohol and deposited in the SMNS collection (Staatliches Museum für Naturkunde Stuttgart)(see Appendix 1). External measurements were made to the nearest 0.01 mm using a stereoscopic microscope and internal features were analysed by a scanning electron microscope. Tadpoles were staged according to Gosner (1960). The terminology for describing external features follows Altig and McDiarmid (1999) and, regarding internal oral structures, Wassersug (1979, 1980).

RESULTS

HYLA LEPTOLINEATA Braun and Braun, 1977

Tadpole description – Our description is based on 17 tadpoles in stages 25 - 37 (Table 1). Body ovoid in dorsal view, more wide than deep, 32% of the total length (Fig. 1). Snout rounded in lateral view, nostrils dorsal and reniform, closer to eye than to tip of snout. Distance between nares 55% of interorbital width. Spiracle sinistral, lateral wall attached to body. Anal tube dextral, right wall displaced anteriorly and dorsally. Tail approximately 68% of total length. Tail fins convex, height aproximatelly 20% of its length. Tail fin ending in an acuminate tip. Fin origin slightly anterior to base of the tail muscle.

Oral disc ventral, width equal to 36% of body width, with lateral folds (Fig. 2). Labial tooth row formula (LTRF) 2(2)/3(1). Labial teeth small, closely positioned. Teeth smaller in A2 than A1, and in P3 than P2. A row of marginal papillae around the whole oral disc, except for a rostral gap. A row of submarginal papillae laterally and ventrally. Beak moderately developed. Upper jaw sheath concave, lower jaw sheath U-shaped, both serrated.

In preserved specimens, body colour greyish to brownish, with notable dark patches and small dots uniformly distributed on dorsum. Tail yellowish, with a blackish to brownish line on the medium portion of the tail muscle. Brownish spots scattered on superior portion of tail. Tail fins transparent. Gut visible through belly skin. Some larvae with darker coloration and greyish to brownish tail fins, dark brownish dorsum and tail muscle.

Oral cavity – Buccal roof elongate with both prenarial and postnarial arenas clearly visible (Fig. 3A) . Prenarial arena with a V-shaped ridge, with a depression at the central portion, placed on the middle width of the arena. Narial valve projection obliquely oriented, with posterior wall higher than anterior wall. Two pronounced lateral ridge papillae, one at each side, and smaller pointed papillae present. Median ridge approximately 75% of the total width of the buccal area. U-shaped buccal roof arena delimited by finger-like papillae with variable length, containing numerous small pustulations. Lateral walls of the buccal roof arena with a row of papillae on each side and pustulations. Glandular zone concentrated centrally. Dorsal velum clearly delimited.

Buccal floor triangulate, with a semicircular posterior limit (Fig. 3B). Two compressed infralabial papillae with ramifications on the borders. Two equally sized lingual papillae, symmetrically arranged in the middle of the tongue. Two diagonal rows of papillae at each side of the buccal floor arena. Small pustulations covering the arena. Ventral velum clearly visible.

DISCUSSION

As Eterovick et al. (2002) suggested, the tadpoles of the species in the *H. polytaenia* group may be distinguished by their tooth row formula. *Hyla polyatenia* has LTRF 2(2)/3(1,2) (Heyer et al., 1990), *H. cipoensis* 2(2)/3(1) and *H. goiana* 2(1,2)/3(1) (Eterovick et al., 2002). Although the tadpole of *H. leptolineata* presents the same LTRF as *H. cipoensis*, 2(2)/3(1), the position of the marginal papillae is different. *Hyla cipoensis* has a single row of marginal papillae on the upper and lower lips, presenting a rostral gap and two rows laterally, while *H. leptolineata* has a row of marginal papillae on the upper lip, also with a rostral gap, and two rows of papillae laterally and on the lower lip.

The distribution ranges of the species in the *H. polytaenia* group seem to be geographically separated. *Hyla leptolineata* is the only species occurring in southern Brazil, in higher regions of the Rio Grande do Sul and Santa Catarina states (Cruz and Caramaschi, 1998; Kwet and Di-Bernardo, 1999). *Hyla goiana* occurs in central-western Brazil, in the Goiás and Distrito Federal states and in the southwest of Minas Gerais. *Hyla cipoensis* and *H. polytaenia* might have an allopatric distribution in southeastern Brazil. Whereas *H. cipoensis* inhabits the higher regions of Serra do Cipó, *H. polytaenia* is found on lower parts of Serra do Mar and Serra da Mantiqueira (Cruz and Caramaschi, 1998).

The adults of *H. leptolineata* inhabit open areas, where they are frequently found in the proximity to small streams and ponds (Kwet, 2001). Only limited data on the tadpole ecology of *H. leptolineata* and the other known species of the *polytaenia* group are available. Tadpoles of *H. leptolineata* were observed throughout the year at the bottom of water bodies among stones (Kwet and Di-Bernardo, 1999). Tadpoles of *H. cipoensis* were observed in small streams and backwaters (Haddad et al., 1988; Eterovick et al., 2002), whereas *Hyla polytaenia* tadpoles were found on the floor of permanent or temporary ponds (Cardoso et al., 1989; Heyer, 1990) The tadpoles of

H. goiana live at the bottom of small streams surrounded by forest vegetation (Eterovick et al., 2002). All these observations about substrate utilization indicate that the tadpoles in this group belong to the benthic morphotype.

ACKNOWLEDGMENTS

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APPENDIX 1

Specimens examined. *Hyla leptolineata* – SMNS9377, SMNS9381, SMNS9383, SMNS9458, SMNS9466, SMNS9478, SMNS9509. The specimens with the number SMNS 9381 were dissected for the study of the internal oral morphology. Some damaged exemplars were not included in the morphometrical analysis.

TABLE 1. Measurements (mean, in millimeters) of tadpoles of *Hyla leptolineata*.

Stage	25	26	28	34	35	36	37
	N= 3	N= 2	N= 3	N=1	N= 1	N= 4	N = 3
Total length	28,67	24,85	41,28	49,85	48,46	46,56	45,44
Body length	10,31	8,62	12,36	14,92	14,92	14,54	13,85
Body width	6,87	5,54	7,18	9,85	10,08	8,85	8,97
Body height	6,00	5,46	6,62	7,85	8,92	7,44	7,38
Tail length	18,36	16,23	28,92	34,92	33,54	32,02	31,59
Eye diameter	1,00	1,08	1,23	1,69	1,69	1,81	1,59
Oral disc width	2,56	1,88	2,79	3,23	3,31	3,37	2,92
Inter orbital distance	3,92	3,23	4,28	5,08	5,38	5,27	4,92
Inter narial distance	2,10	1,69	2,67	2,92	2,92	2,85	2,51
Eye nostril distance	1,87	1,65	1,79	2,31	2,77	2,46	2,46
Nostril snout distance	2,03	2,12	2,10	2,38	2,31	2,35	2,31

The measurements are based on 17 tadpoles in stage 25-37.

Fig. 1

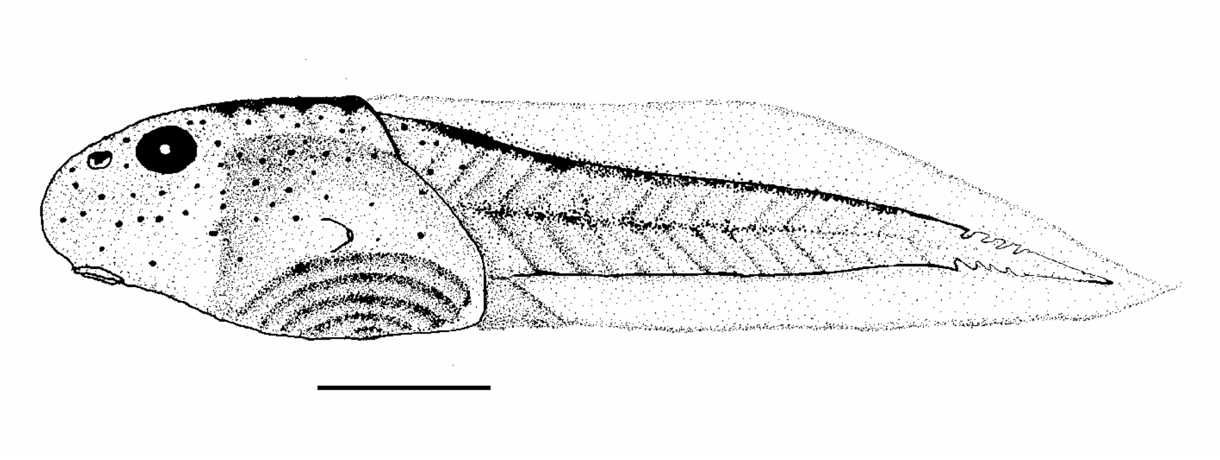


Fig. 2

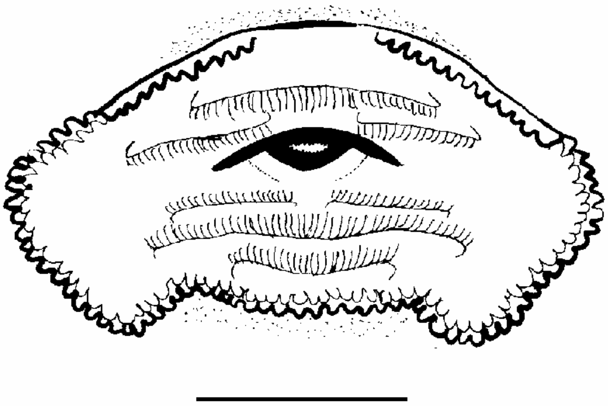
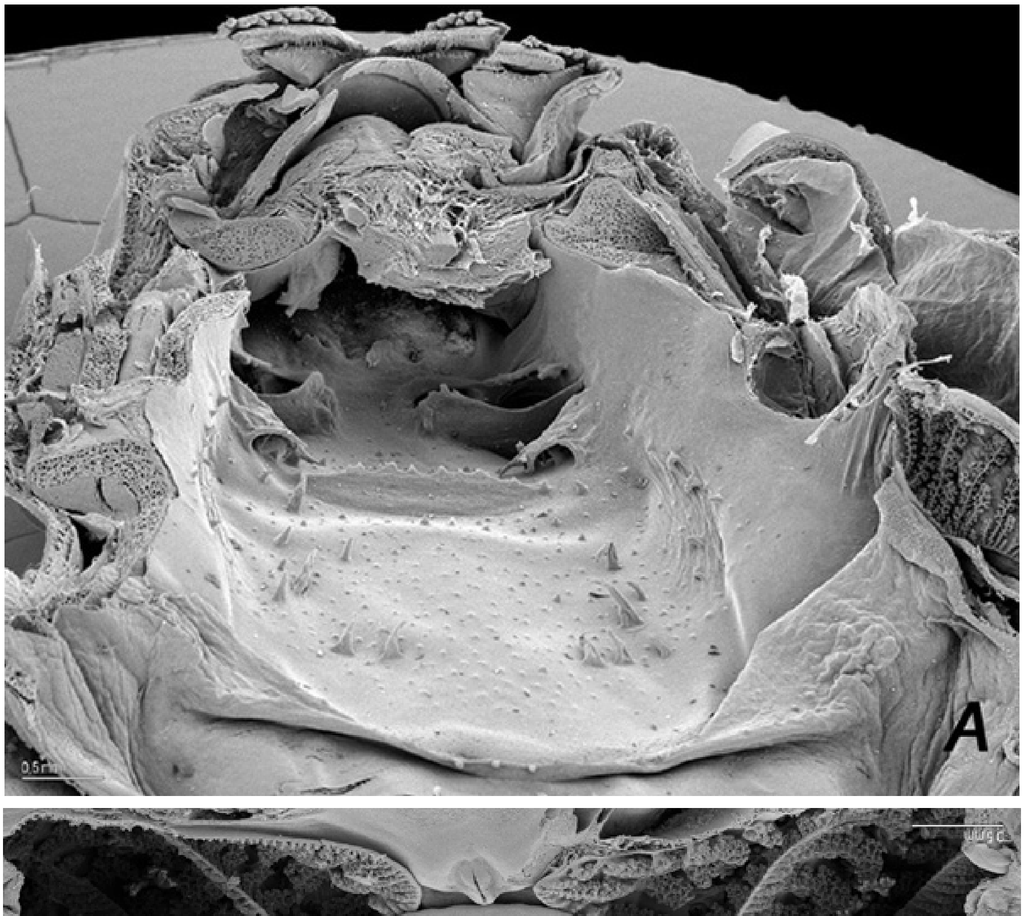


Fig. 3



5.7. Carvalho, Geraldo[1], **Solé, Mirco[2]** & Kwet, Axel[3]

Descrição do girino de *Leptodactylus plaumanni* AHL, 1936 (Anura, Leptodactylidae) do Centro de Pesquisa e Conservação da Natureza Pró-Mata, Rio Grande do Sul, Brasil

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Caderno de Pesquisa série Biologia (2004, eingereicht)

Die Gattung *Leptodactylus* umfasst 65 beschriebene Arten, von denen 9 im Bundesstaat Rio Grande do Sul vorkommen. *Leptodactylus plaumanni*, der bis vor kurzem noch unter dem Synonym *L. geminus* bekannt war, bewohnt die Bundesstaaten Santa Catarina und Rio Grande do Sul in Südbrasilien, sowie die Provinz Misiones in Argentinien. Kaulquappen dieser Art wurden im Waldschutzgebiet Pró-Mata gesammelt, um die externe Morphologie zu untersuchen. Der ovale Körper der Larven macht 35% der Gesamtlänge aus. Das Spirakel befindet sich an der linken Körperseite. Das Mundfeld weist die Zahnformel 1,1-1/1-1,2 und eine einzelne Papillenreihe auf. Die Grundfarbe der Larven reicht von Braun bis Dunkelgelb, die Bauchseite ist heller. Die Umrisse des Darms schimmern durch die Haut. Die Schwanzmuskulatur ist gut entwickelt, der Schwanz ist mit kleinen schwarzen Flecken gesäumt. Die Kaulquappen der Gattung

Leptodactylus sehen sich einander recht ähnlich. Auch die Zahnformel ermöglicht in den meisten Fällen keine sichere Bestimmung.

DESCRIPTION OF THE TADPOLE OF *Leptodactylus plaumanni* AHL, 1936 (ANURA, LEPTODACTYLIDAE) FROM CENTRO DE PESQUISAS E CONSERVAÇÃO DA NATUREZA PRÓ-MATA, RIO GRANDE DO SUL, BRAZIL

Geraldo Carvalho[1,2]
Mirco Solé [2]
Axel Kwet [2,3]

ABSTRACT

The external morphology of the tadpole of *Leptodactylus plaumanni* Ahl, 1936 is described from Rio Grande do Sul state, southern Brazil. We provide a table of measurements, parameters and figures for the differentiation from tadpoles of the other species in this genus distributed in Rio Grande do Sul.

Key words: tadpoles, distribution, *Leptodactylus.*

DESCRIÇÃO DO GIRINO DE *Leptodactylus plaumanni* AHL, 1936 (ANURA, LEPTODACTYLIDAE) DO CENTRO DE PESQUISA E CONSERVAÇÃO DA NATUREZA PRÓ-MATA, RIO GRANDE DO SUL, BRASIL.

RESUMO

A morfologia externa do girino de *Leptodactylus plaumanni* Ahl, 1936 está descrito, coletado no Rio Grande do Sul - Brasil. O presente trabalho apresenta uma tabela de medidas e parâmetros, bem como figuras para a diferenciação do girino dos outros do mesmo gênero ocorrentes no Estado do Rio Grande do Sul, Brasil.

Palavras chaves: girinos, distribuição, *Leptodactylus.*

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INTRODUÇÃO

O gênero *Leptodactylus* contém atualmente 65 espécies (FROST, 2002), sendo que nove ocorrem no Rio Grande do Sul (KWET, 2001). Excetuando *L. plaumanni*, com apenas algumas observações sobre seus girinos (SOLÉ e KWET, 2002), e *L. elenae*, ainda sem descrição, a morfologia larval de algumas espécies deste gênero já foram descritas: *L. mystacinus* e *L. chaquensis* (CEI, 1980); *L. ocellatus*, *L. gracilis* e *L. latinasus* (= *L. prognathus*) (FERNÁNDEZ e FERNÁNDEZ, 1921); *L. fuscus* (LESCURE, 1972); e *L. labyrinthicus* (VIZOTTO, 1967).

Leptodactylus plaumanni AHL, 1936 (= *L. geminus* BARRIO, 1973) é conhecido ao norte da província de Missiones, na Argentina, e nos estados do Rio Grande do Sul e de Santa Catarina no sul do Brasil (FROST, 2002). Nessas mesmas regiões é encontrada *L. gracilis* (DUMÉRIL e BIBRON, 1841), que apresenta marcante semelhança morfológica com *L. plaumanni*. SCROCCHI e LAVILLA (1986) propõem medidas morfométricas com validade somente para indivíduos adultos, sem eficácia para indivíduos jovens. As espécies podem ser distinguidas em habitat natural pelas características de suas vocalizações, conforme BARRIO (1973) e KWET (2001). A taxonomia e as semelhanças entre essas espécies foram discutidas por BARRIO (1973), HEYER (1978), CEI (1980), SCHROCHI e LAVILLA (1986) e KWET et al. (2001).

Observações sobre a biologia reprodutiva e os girinos de *L. plaumanni* foram reportadas por KWET e DI-BERNARDO (1999), mas a morfologia larval ainda é desconhecida e abordada no presente estudo.

MATERIAIS E MÉTODOS

As larvas de *Leptodactylus plaumanni* foram coletadas no Centro de Pesquisas e Conservação da Natureza Pró-Mata, localizado ao nordeste do Estado do Rio Grande do Sul, latitude 29º27’ a 29º35’ S e longitude 50º08’a 50º15’ W, Brasil. Os exemplares coletados foram conservados em álcool 70% e depositados nas coleções do Staatliches Musem für Naturkunde Stuttgart (SMNS) (Lotes SMNS 9316, SMNS 9556 e SMNS 9557; coletados entre novembro de 1995 e janeiro de 1996 por Axel Kwet e Marcos Di-Bernardo) e do Museu de Ciências e Tecnologia (MCT) da Pontifícia Universidade Católica do Rio Grande do Sul (Lote MCP 3897, coletado em janeiro de 2001 por Mirco Solé e e Marcos Di-Bernardo).

As medidas foram tomadas com auxílio de estereomicroscópio com definição aproximada de 0,01 mm. A terminologia utilizada segue ALTIG (1970) e os estágios foram identificados de acordo com GOSNER (1960).

RESULTADOS

Leptodactylus plaumanni Ahl, 1936, descrição de girinos no estágio 23 ao 42 segundo Gosner, 1960 (Tab.1):

Em todos os estágios, o corpo é ovóide em vista lateral, elíptico em vista dorsal, mais largo que alto, representando 35% do comprimento total. Focinho arredondado em vista dorsal. Narinas arredondadas, localizadas no meio caminho entre o olho e o focinho ou 1/5 menor que a distância até o olho. Distância entre as narinas é igual a 79% da distância interorbital. Espiráculo sinistral, parede lateral livre. Tubo anal medial.

Cauda aproximadamente 65% do comprimento total. Nadadeiras caudais estreitas, com origem da nadadeira dorsal ligeiramente anterior à junção entre o músculo caudal e o corpo.

Disco oral antero-ventral, fórmula dentária 1, 1-1/1-1, 2. Fileira única de papilas, dispostas entremeadas, muito agrupadas nas laterais e interrompidas no lábio superior. Bico córneo queratinizado. Mandíbula superior côncava, mandíbula inferior em formato de “V”, ambas serrilhadas.

Em espécimes preservados, dorso castanho a amarelo escuro, ventre de coloração escura por apresentar o intestino por transparência, região gular amarela. Cauda com musculatura bem definida amarela ou amarronzada e nadadeiras em tom amarelado transparente, com pequenas pintas marrons nos indivíduos em estágio mais avançado de desenvolvimento.

1. DISCUSSÃO

Os girinos do gênero *Leptodactylus* compartilham características morfológicas como disco oral antero-ventral, olhos dorsais, fileira única de papilas marginais interrompidas na região medial superior, papilas submarginais ausentes, narinas mais perto do disco oral que dos olhos, espiráculo sinistral, maxila com arco comprido e uniforme, mandíbula em forma de U ou V, abertura anal medial, membrana dorsal da cauda estreito, iniciando próxima a junção entre o músculo caudal e o corpo, cauda na ápice redundado até pontiagudo, corpo em vista dorsal oval e em vista lateral dorso-ventralmente achatado, comprimento na fase larval 36 entre 25-60mm, comprimento da cauda variando de 5 a 7 décimos do comprimento total (CEI, 1980; MCDIARMID e ALTIG, 1999).

Em diversas publicações a formula dentária esta sendo citado como possibilidade de diferencia algumas espécies do gênero Leptodactylus. De acordo com a literatura examinada a única espécie do gênero *Leptodactylus* ocorrente no Rio Grande do Sul que pode ser identificada usando somente a fórmula dentária é *L. labyrinthicus*, apresentando 1 / 1-1, 1 (CEI, 1980). *L. gracilis* e *L. plaumanni* apresentam conforme SOLÉ & KWET (2002) a mesma variação da fórmula dentária, podendo ocorrer nas duas espécies tanto a fórmula 1, 1-1 / 1-1, 2 como 1, 1-1 / 3. Além destas, a espécie *L. latinasus* também apresenta fórmula dentária 1, 1-1 / 3 (FERNÁNDEZ e FERNÁNDEZ, 1921), bem como *L. mystacinus* e *L. fuscus* que apresentam a fórmula dentária 1, 1-1 / 1-1, 2 (CEI, 1980; LESCURE 1972). *L. ocellatus* e *L. chaquensis* formam um grupo morfológico separado apresentando fórmula dentária 2 / 3 (CEI, 1980).

Os hábitos reprodutivos das espécies do gênero *Leptodactylus* registrados para o Rio Grande do Sul são semelhantes, sendo os ninhos de espuma, na maioria subterrâneos com exceção de *L. chaquensis, L. ocellatus* e *L. labyrinthicus*, que apresentam ninhos de espuma na superfície de água parada. As desovas são registradas para os períodos de setembro a fevereiro. O número de ovos varia de 150-300 ovos grandes em *L. gracilis* e *L. plaumanni* a milhares de ovos pequenos em *L. chaquensis*, *L. latinasus, L. labyrinthicus* e *L. ocellatus* (CEI, 1980; FERNÁNDEZ e FERNÁNDEZ, 1921 e KWET e DI-BERNARDO, 1999)

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Tabela 1: Medidas dos girinos de *Leptodactylus plaumanni* Ahl, 1936 utilizados para a descrição.

Est./N° Indiv.	23/1	24/1	25/12	26/3	27/2	28/1	30/1	32/1	34/2	35/1	36/2	42/1
Comp. Total	6.99	6.84	11.09	13.42	13.13	20.67	22.69	24.09	25.88	31.86	30.61	33.10
Comp. Da cauda	4.20	4.04	7.23	8.70	8.63	12.59	13.99	15.23	16.48	21.29	20.21	20.36
Comp. Do corpo	2.80	2.80	3.86	4.71	4.51	8.08	8.70	8.86	9.40	10.57	10.42	12.74
Larg. Do corpo	1.86	1.71	2.24	3.06	2.72	4.66	4.66	4.35	5.75	6.68	6.30	6.37
Alt. do corpo	1.55	1.86	2.06	2.74	2.49	3.89	4.35	3.89	4.97	5.75	6.06	4.97
Alt. da cauda	1.40	1.71	1.99	2.44	2.49	2.95	3.57	3.73	4.90	5.75	5.29	4.82
Diam. Do olho	0.31	0.31	0.47	0.47	0.47	0.78	0.93	0.93	0.93	0.93	0.93	1.24
Dist. Interocular	0.31	0.31	0.59	0.78	0.62	1.55	1.71	1.71	1.94	1.86	2.02	2.18
Dist. Internasal	0.16	0.31	0.47	0.62	0.62	1.09	1.24	1.24	1.48	1.55	1.24	1.71
Dist. Nar. Olho	0.16	0.16	0.47	0.62	0.62	0.93	0.93	1.09	1.01	1.09	1.09	1.40
Dist. Nar. Focinho	0.16	0.16	0.31	0.47	0.47	0.78	0.78	0.78	0.78	0.93	0.78	1.24

Est – Estágio segundo Gosner (1960).

Figura 1: Girino de *L. plaumanni*

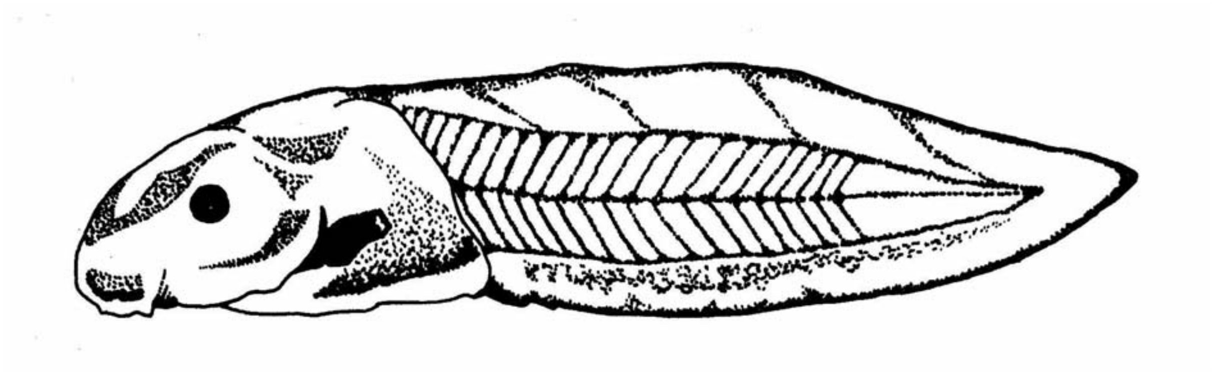
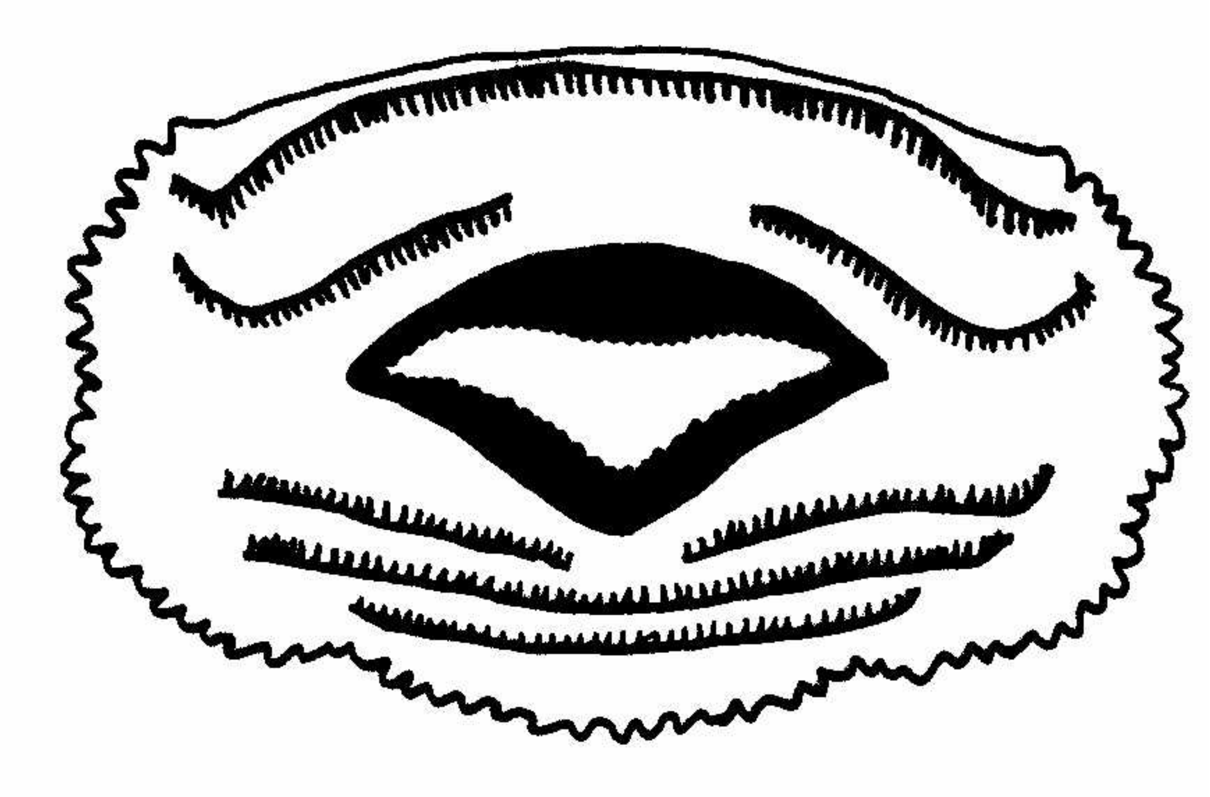


Figura 2: Boca do girino de *L. plaumanni*



5.8. Miranda, Tatiana[1]; Ebner, Martin[2]; **Solé, Mirco[3]** & Kwet, Axel[4]

Estimativa populacional de *Pseudis cardosoi* (Anura, Hylidae), com emprego de Método Fotográfico para reconhecimento individual

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Mit dieser Untersuchung stellen wir eine nicht-invasive Alternative zur Phalangenamputation vor. Während einer Populationsstudie über den Harlekinfrosch *Pseudis cardosoi* auf dem Araukarienplateau bemerkten wir, dass alle Tiere eine individuelle Färbung der Oberschenkel aufwiesen. Diese Muster wurden fotografiert und in einer Kartei abgelegt. Es wurden 49 Männchen, 31 Weibchen und 30 Jungtiere gefangen und fotografiert. Die Wiederfangrate war gering: 13 Männchen (27 %), acht Weibchen (26%) und fünf Juvenile (17 %) konnten bis zu fünf mal wiedergefangen werden. Die Muster auf den Oberschenkeln werden schon während der Metamorphose angelegt, sie verändern sich während des späteren Wachstums nicht mehr. Die Größe der untersuchten Population wurde auf 130 Individuen geschätzt. Die fotografische Methode erwies sich als zuverlässig, dürfte aber nur bei kleinen Populationen erfolgreich anwendbar sein.

Estimativa populacional de *Pseudis cardosoi* (Anura, Hylidae), com emprego de Método Fotográfico para reconhecimento individual

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RESUMO

Um estudo populacional de *Pseudis cardosoi* KWET, 2000 foi realizado ao longo de 11 meses, na Fazenda Três Estrelas, localizada no município de São Francisco de Paula, RS, empregando-se um método fotográfico para reconhecimento individual. Este método consiste em fotografar as marcas naturais presentes na face interna das coxas de *P. cardosoi*, que são únicas para cada indivíduo da população. Estas marcas estão presentes em machos e fêmeas e são invariáveis ao longo da metamorfose e da fase adulta. Foram fotografados 49 machos, 31 fêmeas e 30 juvenis. O método proporcionou reconhecimento individual inequívoco e a freqüência de recapturas foi alta: 13 machos (26,5%), oito fêmeas (25,8%) e cinco juvenis: (16,7%). Através do método Jolly-Seber, a população local foi estimada em 129,5 indivíduos.

Palavras chave: Hylidae, *Pseudis cardosoi*, estudo populacional, método fotográfico, marcas naturais.

ABSTRACT

Estimation of population size in *Pseudis cardosoi* (Anura, Hylidae), using photoidentification as a method for individual recognition.

A population study in *Pseudis cardosoi* KWET, 2000 was carried out during 11 months at “Três Estrelas” farm in the municipality of São Francisco de Paula, RS, using a photographical method for individual recognition. This method is based on recording of the thigh colour pattern of the inner part of the hind legs in *P. cardosoi*, which is unique for each individual in the population. This unique thigh colour pattern is found in males and females, not changing during the metamorphosis or during the adult life stage. 49 males, 31 females and 30 juveniles were photographed, and the method allowed unambiguous individual recognition. The frequency of recaptures was high: 13 males (26.5 %), 8 females (25.8 %) and 5 juveniles (16.7 %). Using the Jolly-Seber method, the local population was estimated to consist of 129,5 individuals.

Key words: Hylidae, *Pseudis cardosoi*, population study, photographic method, natural marks.

INTRODUÇÃO

Estudos recentes no Centro de Pesquisas e Conservação da Natureza Pró-Mata (CPCN Pró-Mata), situado no limite sudeste da Floresta com Araucária, mostraram que quase um terço das 36 espécies encontradas constituíram espécies novas, espécies revalidadas, ou registros novos para o Rio Grande do Sul (KWET & DI-BERNARDO, 1999; KWET, 2001). Dentre as espécies descritas para esta área encontra-se *Pseudis cardosoi* KWET, 2000, anfíbio anuro recentemente incluído na família Hylidae como subfamília Pseudinae (DUELLMAN, 2001). Esta proposta foi questionada por BRANDÃO et al. (2003) que trata os Pseudinae como família própria (Pseudidae). DARST & CANNATELLA (2004) sugeriram que Pseudinae está inserido em Hylidae (Hylinae), sugerindo a sinonimização não só de Pseudidae, mas também de Pseudinae com Hylinae. Neste trabalho nós adotamos DARST & CANNATELLA, 2004 tratando os “Pseudinae” como Hylidae. O gênero *Pseudis* é composto por espécies exclusivamente aquáticas, que ocorrem na América do Sul a leste dos Andes, desde a Venezuela até o leste da Argentina e Uruguai (FROST, 2004). A distribuição de *Pseudis cardosoi* abrange as áreas do Planalto das Araucárias nos estados do Rio Grande do Sul e Santa Catarina. Caracteriza-se pelo macho exibir dois sacos vocais (KWET, 2000), um dimorfismo sexual evidente, que auxilia na identificação dos sexos.

Existem diferentes métodos de marcação e recaptura para estudos populacionais, e o método fotográfico pode ser uma alternativa não ofensiva em relação aos métodos que empregam a amputação de falanges (“toe clipping”, HERO, 1989; DONNELLY et al., 1994), ou outros métodos invasivos (MEYER & GROSSE, 1997; STREICH et al., 1997). A amputação de falanges foi o método de marcação dominante para anfíbios até o início dos anos 90, ainda sendo usado atualmente (BOOMSMA & ARNTZEN, 1985; TEJEDO, 1988; SINSCH, 1992; KUHN, 1994; VAN GELDER & STRIJBOSCH, 1996).

Certos anfíbios apresentam padrões de desenhos que são únicos para cada indivíduo, distinguindo-os dos outros membros da população. Métodos que dão ênfase a estes padrões de desenhos naturais podem ser empregados em geral (WINKLER & HEUNISCH, 1997). Para anfíbios anuros, estes métodos não são normalmente usados, embora esta metodologia possa ser necessária (McCARTHY & PARRIS, 2004).

Através de estudos preliminares, observamos que todos indivíduos da espécie *Pseudis cardosoi* apresentam um desenho na face interna das coxas de ambas as pernas, que é único para cada animal, e que funciona como uma “impressão digital”. Percebemos que este desenho poderia ser usado como uma marca natural, e que o método não causaria dano físico nos exemplares registrados perante os outros membros da população, que não foram marcados.

Este estudo objetivou apresentar um novo método de marcação para anfíbios anuros, estimar o tamanho da população de *P. cardosoi* em quatro poças situadas no Planalto das Araucárias do Rio Grande do Sul e verificar se nestas poças as populações eram isoladas ou uma população aberta.

MATERIAL E MÉTODOS

O trabalho de campo foi desenvolvido na Fazenda Três Estrelas, 923 m acima do nível do mar. Esta área é próxima ao CPCN Pró-Mata, localizado entre os paralelos 29º27’e 29º35’S e os meridianos 50º08’e 50º15’W, no município de São Francisco de Paula, Rio Grande do Sul (BERTOLETTI & TEIXEIRA, 1995). Os estudos foram realizados no período de 17.02 a 02.12.2003, com excursões mensais a campo. As coletas foram realizadas em quatro poças identificadas como B1 (23,5 x 7,7 m), B2 (36 x 16 m), B3 (27 x 25 m) e B4 (30 x 18 m). A distância entre B1 e B2 foi de 15,5 m; as poças B2 e B3 permaneceram ligadas por um canal durante a época de chuvas, e a distância entre elas foi de 13,5 m. A distância entre B3 e B4 foi de 52 m, entre B1 e B3 de 29 m, entre B1 e B4 de 83,5 m e entre B2 e B4 de 58,1 m.

Os anuros foram capturados no campo, armazenados em baldes com água e levados ao laboratório. O método de reconhecimento individual consistiu em fotografar as coxas de cada espécime, onde estão os desenhos com linhas, manchas e pontos, usando câmara fotográfica digital Caplio RR30, com 3.2 mega pixels e zoom óptico. Cada indivíduo foi posicionado com o ventre voltado para cima, sobre fundo contrastante, com uma etiqueta contendo os dados de coleta (local, data e código do indivíduo) e fotografado. A câmara fotográfica foi fixada de modo a permitir que cada foto fosse tirada sempre a uma mesma distância (12 cm) (Fig. 1). Depois de fotografados os hilídeos coletados foram devolvidos para as poças onde foram capturados. As fotos foram transferidas para um computador, impressas e coladas na ficha individual de cada exemplar, e um álbum digital foi elaborado. As fichas continham dados sobre a coleta e sobre o espécime capturado (local de captura, coletores, número de campo, número da captura, código, data da coleta, data da soltura, hora da coleta, temperatura do ar, temperatura da água, umidade do ar, número de campo, idade, sexo, dados sobre a atividade, comprimento rostro-cloacal (CRC), massa, observações). Para o cálculo do tamanho da população (N_i) e desvio padrão ($SE\ N_i$), usou-se o método Jolly-Seber (DONNELLY & GUYER, 1994).

$$N_i = \frac{M_i\,(n_i+1)}{(m_i+1)}$$

$$SE\ N_i = \left\{ N_i\ (N_i - n_i)\left[\frac{M_i - m_i + r_i}{Mi}\left(\frac{1}{y_i} + \frac{1}{\mathrm{r_i}} \right) + \frac{1}{m_i} + \frac{1}{n_i} \right]\right\}$$

Onde Mi é o número de animais registrados em risco no dia i (animais em perigo de vida ou de extravio durante o estudo populacional); n_i é o número total de animais capturados no dia i; m_i é o número total de animais registrados e coletados no dia i; r_i é o número total de animais soltos no dia i; y_i é o número de indivíduos registrados e soltos no dia i e capturados depois do dia i (HEYER et al. 1994).

O reconhecimento dos espécimes recapturados foi feito no laboratório, através da comparação do desenho das coxas direita e esquerda dos indivíduos recém capturados e fotografados, com as fotos digitais dos animais já existentes no álbum. Para elaborar o álbum digital utilizou-se o programa Microsoft Word for Windows XP. As fotos de todos os animais capturados foram divididas em arquivos de acordo com o sexo, idade e padrão de pigmentação das coxas (linhas, manchas e pontos). Para facilitar a identificação, os desenhos foram considerados da seguinte maneira: a linha central foi considerada linha 0, as abaixo desta foram consideradas, -1,-2 ... e as acima desta foram consideradas +1,+2 ... (Fig. 2). As fotos foram divididas com base nestes desenhos, por exemplo, se a linha 0 e/ou as outras eram inteiras ou seccionadas, se estavam presentes ou ausentes, se haviam pontos ou manchas além das linhas.

RESULTADOS

No período do experimento foram coletados 110 animais: 49 machos, 31 fêmeas e 30 juvenis, que foram identificados individualmente com base nio padrão de manchas, linhas e pontos existentes na parte interna das coxas. Este método de reconhecimento simples foi aplicável nos machos, fêmeas e nos juvenis porque todos mostraram o padrão de desenhos nas coxas de ambas as pernas. A documentação dos indivíduos pré e pós-metamorfoseados possibilitou observar que as marcas nas coxas dos animais desta espécie estão definidas antes da metamorfose se completar (Fig. 3a-b), se mantendo estáveis até o animal atingir a idade adulta (4a-d).

A grande variabilidade dos desenhos nas coxas proporcionou identificação inequívoca, por isso os animais marcados tiveram 100% de reconhecimento quando recapturados.

A população estimada foi $N = 129{,}5$ com desvio padrão $SE\ N = 75{,}5$. A freqüência de recapturas mostrou-se alta, sendo que a maioria dos animais foi capturada apenas uma vez. Alguns machos e fêmeas foram recapturados até quatro vezes e os juvenis, embora tenham sido a categoria com menor número de indivíduos coletados (n=30), foram recapturados até cinco vezes (Fig. 5). Entre os animais registrados foram recapturados 13 machos (26,5%), oito fêmeas (25,8%) e cinco juvenis (16,7%) (Fig. 6).

Registrou-se deslocamento entre as poças pelos animais identificados pelos números 2 (B1 para B2), 4 (B1 para B2), 20 (B1 para B2 e volta para B1), 35 (B2 para B1), 51 (B1 para B2) e 72 (B4 para B1).

A efetividade deste método é demonstrada com o indivíduo número 11 da poça B1, que foi coletado cinco vezes durante o período do estudo, sendo juvenil na primeira e segunda capturas (Fig. 4a-d).

DISCUSSÃO & CONCLUSÃO

Em estudos populacionais, os modelos de marcação e recaptura assumem que: (1) a amostra tomada inicialmente é representativa da população inteira (não baseada em idade ou sexo); (2) todos os animais capturados na amostra inicial são marcados e as marcas têm que ser permanentes e registradas corretamente; (3) os animais marcados são soltos e distribuídos aleatoriamente na população; (4) a marcação não afeta a probabilidade de recaptura ou sobrevivência (ou seja, animais marcados e não marcados têm a mesma probabilidade de serem capturados). Para que um trabalho com parâmetros populacionais apresente bons resultados, é preciso observar que nenhum destes pré-requisitos seja violado (DONNELLY et al., 1994). Este método inicialmente usado como controle para o método "toe clipping", (HERO, 1989; DONNELLY et al., 1994) mostrou-se mais eficiente. O método "toe clipping" apresentou algumas falhas por que *Pseudis cardosoi* perde facilmente a primeira falange dos dedos, que se regenera facilmente (obs. pess.). Não está esclarecido como *P. cardosoi* perde a primeira falange dos dedos, mas supomos que isso possa acontecer em ataques de predadores.

O método fotográfico foi de fácil execução. Cada foto demorou cerca de um minuto para ser feita. O método foi inequívoco em relação aos animais marcados e o número de recapturas foi grande (Fig. 6). O número de recapturas foi limitado por alguns fatores tais como: a) alto índice de predação nas poças estudadas, por estarem em campo limpo; b) pelo campo sofrer queimadas freqüentes; c) por a população estudada ser aberta, d) pela flutuação da população durante as coletas em diferentes épocas do ano. O hábito aquático ou fossorial pode dificultar a freqüência de recapturas (HEYER et al., 1994).

A freqüência de recapturas de machos e fêmeas foi parecida, mas os juvenis apresentaram freqüência menor (Fig. 5). Isto pode ter ocorrido porque anfíbios anuros são muito sensíveis a fatores externos e a predação durante a fase da metamorfose e no estágio juvenil (DUELLMAN & TRUEB, 1986; HEYER et al., 1994).

Estudos de populações usando métodos fotográficos têm sido praticados na última década (e.g., WINKLER & HEUNISCH, 1997; MEYER & GROSSE, 1997; STREICH et al., 1997), porém a maioria dos animais estudados apresentou mudanças na pigmentação fotografada e/ou a pigmentação estava presente apenas nos machos, o que levou os pesquisadores a fotografarem outra parte do corpo do animal como controle. No estudo de populações de *P. cardosoi* não ocorreram estes problemas, pois as manchas permaneceram inalteradas desde a metamorfose (Fig. 3a-b), e estiveram presentes tanto em machos quanto em fêmeas (Fig. 3a-b, 4a-d).

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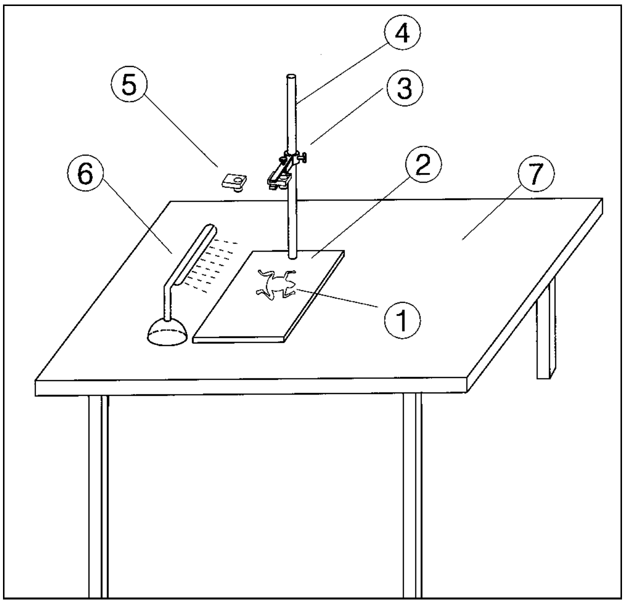


Fig. 1:

Fig. 2

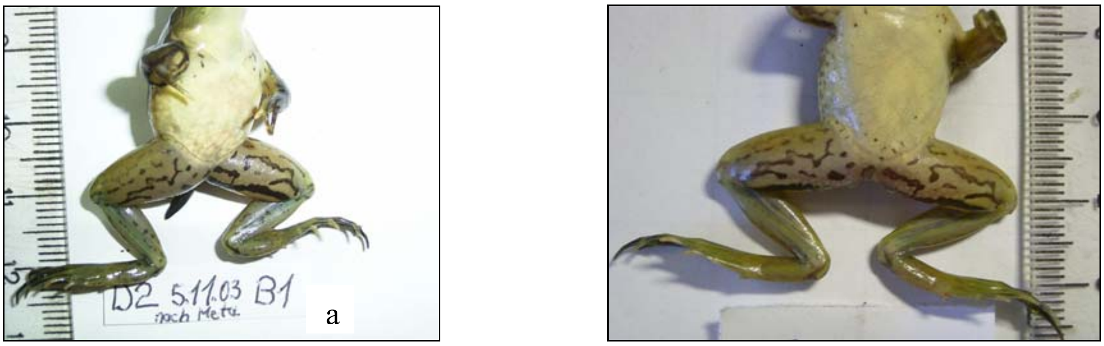
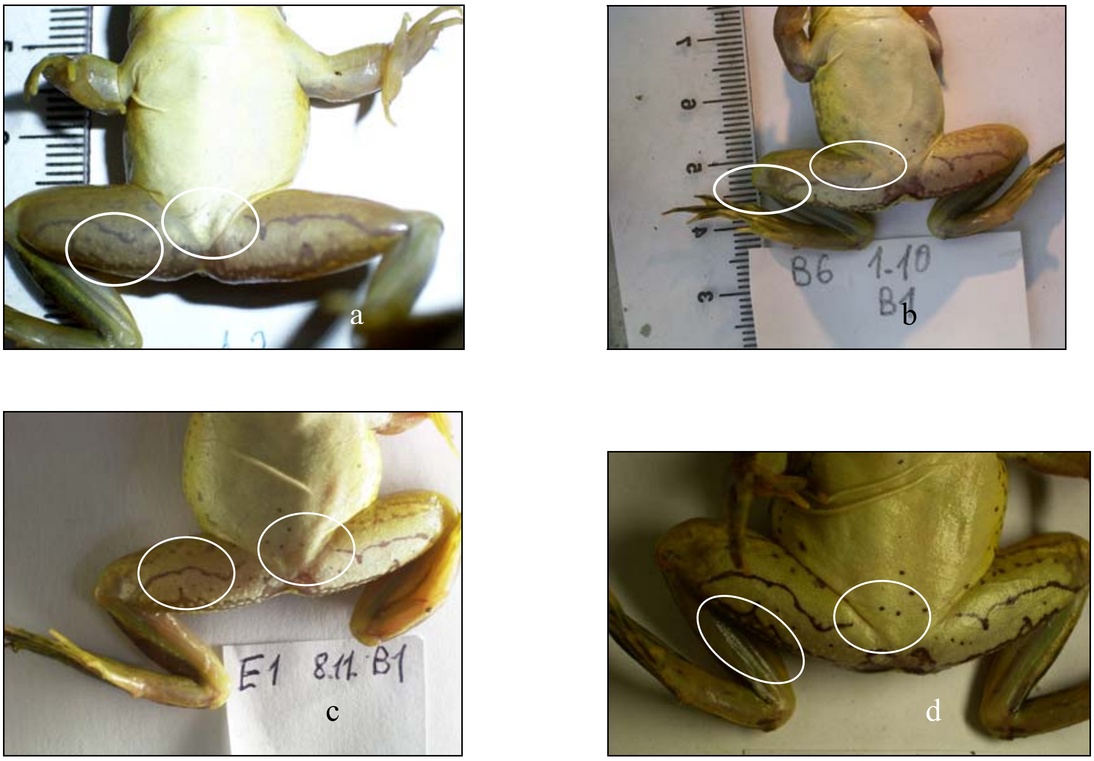


Fig. 3



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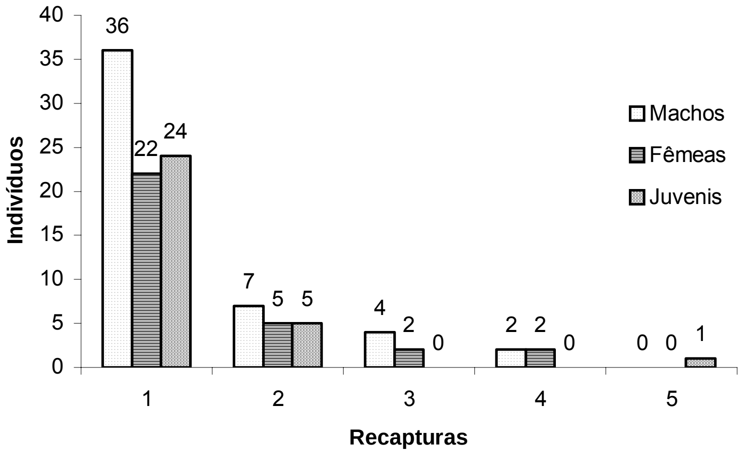

Indivíduos
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Recapturas
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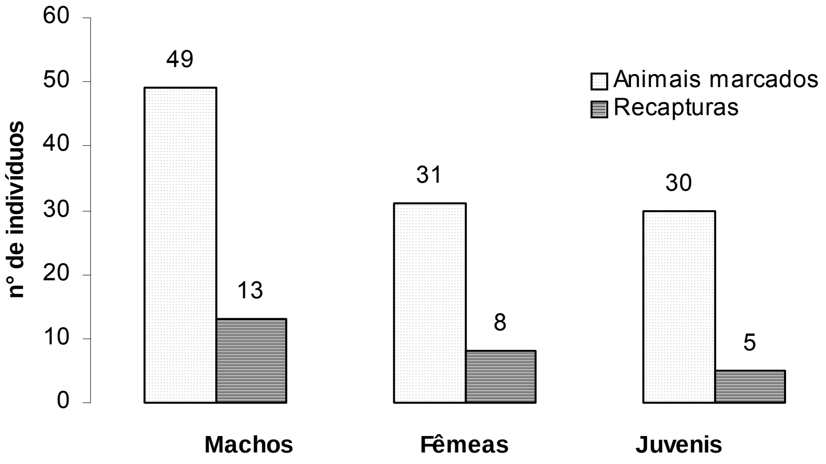
Juvenis

Fig. 5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Animais marcados
Recapturas
Machos
Fêmeas
Juvenis

Fig. 6

Fig. 1: Montagem com a câmara fotográfica digital, para fotografar as coxas de *Pseudis cardosoi*. 1. *Pseudis cardosoi* em posição ventral, 2. fundo contrastante, 3. suporte para câmara fotográfica, 4. barra de sustentação, 5. câmara digital, 6. fonte de luz, 7. mesa de trabalho.

Fig. 2: Método de leitura das linhas de pigmentação das coxas de *Pseudis cardosoi*.

Fig. 3: Exemplar n° 93 de *P. cardosoi*: a. capturado em 5.11.2003, em metamorfose com as manchas nas coxas já definidas; b. recapturado em 02.12.2003, com a metamorfose completa.

Exemplar n°11 de *P. cardosoi*: a. capturado em 04.08.2003, na fase juvenil; b. recapturado em 01.10.2003, com dimorfismo sexual evidente, fêmea; c. recapturado em 08.11.2003, na fase adulta; d. recapturado em 02.12.2003, na fase adulto.

Fig. 5: Freqüências de recapturas de machos, fêmeas e juvenis na Fazenda Três Estrelas.

Fig. 6: Freqüências de recapturas de *Pseudis cardosoi* na Fazenda Três Estrelas, usando o método "padrão das coxas" (machos: n=49; fêmeas: n=31; juvenis: n=30).

5.9. **Solé**, **Mirco**[1] & Kwet, Axel[2]

Liophis jaegeri (Jaeger´s Ground Snake) Diet.

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Herpetological Review (2003) 34: 69.

Von der Buntnatter *Liophis jaegeri* ist bekannt, dass sie sich von kleinen Fröschen und Kaulquappen ernährt. Die meisten Pfeiffrösche sind Schaumnestbauer. Solche Nester schützen die Eier vor Austrocknung, Lichteinfluss und Prädatoren. *Leptodactylus plaumanni* ist ein Pfeiffrosch, der seine Schaumnester in kleinen Höhlen am Gewässerrand anlegt. Wir konnten beobachten, wie eine juvenile *Liophis jaegeri*-Natter mit dem Kopf in einem Schaumnest steckte und sich langsam drehte. Nach dem Fang würgte diese Schlange weißen Schaum und 11 Kaulquappen im Gosner-Stadium 19 aus. In dem verbliebenen Nestrest fanden wir Kaulquappen im selben Entwicklungsstadium. Um unsere Beobachtung zu verifizieren, setzten wir die gefangene Schlange mit vier weiteren in ein Aquarium und boten ihnen ein wenige Stunden vorher gebautes Schaumnest von *L. plaumanni* an. Alle fünf Schlangen begannen, den Schaum zu verschlingen. Es handelt sich um den ersten Nachweis, dass Schlangen auch Schaumnester fressen.

LIOPHIS JAEGERI (Jaeger´s Ground Snake). **DIET.** *Liophis jaegeri* is a small colubrid occurring in Brazil, Bolivia, Paraguay, Argentina, and Uruguay. This snake lives in humid and aquatic habitats where it mainly feeds on amphibians. After a heavy rainfall on 7 January 2001 at 2130 h, we observed a juvenile *L. jaegeri* (226 mm SVL; 277 mm total length; 7.72 g mass) at a small pond in the CPCN Pró-Mata reserve, municipality of São Francisco de Paula, Rio Grande do Sul, southern Brazil (960 m elevation). The snake's head was inside a small hole in the mud and its tail was slowly moving. After being caught, the snake regurgitated white foam and 11 tadpoles at Gosner stage 19. Inside the hole, we found a foam nest of the leptodactylid *Leptodactylus plaumanni* containing tadpoles of the same stage. To our knowledge, this is the first record of a snake feeding on a subterranean anuran foam nest. To verify our observation, this snake and four other *L. jaegeri* from the same area (two adult females, one adult male, and one juvenile) were kept together inside a terrarium. For one week the snakes were only provided with water, then we offered a newbuilt foam nest of *L. plaumanni*. The specimen found eating in the hole under natural conditions immediately approached the nest and began to feed on eggs by totally immersing its head in the foam. After 2 min. another snake started eating, and after 10 min. all five snakes were feeding on the nest. These observations confirm that the diet of *L. jaegeri* comprises anuran eggs and tadpoles from foam nests.

We thank Marcos Di-Bernardo for his cooperation.

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5.10 Kwet, Axel[1] & **Solé**, **Mirco[2]**

Elachistocleis erythrogaster (Redbellied Oval Frog). Defensive Behavior

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Herpetological Review (2002) 33: 46

Elachistocleis erythrogaster ist ein auf dem Araukarienplateau endemischer Vertreter der Engmaulfrösche. Wir beschreiben ein bisher unbekanntes Abwehrverhalten. Dabei füllen gefangene Frösche ihre Lungen, spreizen ihre Beine ab und heben den hinteren Körperteil an. Diese Position wird ca. eine Minute lang gehalten. Die Abwehr-Reaktion kann mehrere Male nacheinander durch Berühren des Frosches ausgelöst werden. In machen Fällen sondern sie auch einen weißen Schleim ab, der möglicherweise toxisch für Prädatoren ist. Obwohl *Elachistocleis erythrogaster* eine kräftig rot gefärbte Bauchseite besitzt, wird diese bei Störung nicht exponiert. Es handelt sich bei dem beobachteten Verhalten deshalb nicht um einen Unkenreflex.

ANURA

ELACHISTOCLEIS ERYTHROGASTER (Red-bellied Oval Frog). **DEFENSIVE BEHAVIOR.** *Elachistocleis erythrogaster*, a small microhylid endemic to the southeastern *Araucaria* plateau of Rio Grande do Sul, southern Brazil, was recently described by Kwet and Di-Bernardo (1998. Stud. Neotrop. Fauna Environ. 33:7–18). On 15 January 2001, six *E. erythrogaster* were collected at a pond near the CPCN Pró-Mata reserve, municipality of São Francisco de Paula, Rio Grande do Sul (960 m elev.). They were carried to the laboratory and kept in a terrarium. On 18 January, one individual was placed on a wooden table to be photographed. Immediately, the frog inflated its lungs, stretched out the hindlimbs and elevated the posterior part of the body. This posture was maintained for about one minute and it was possible to trigger this behavior several times by touching lightly. Of the six specimens tested, five demonstrated this defensive behavior when handled.

The antipredator behavior of presenting a larger image to potential predators is common among heavy-bodied anurans, although this is the first record for a New World microhylid. Puffing up the body and outstretching the limbs makes *E. erythrogaster* larger and should help protect against ingestion. Additionally, this microhylid produces a whitish, sticky skin secretion that might be toxic to predators. When handled, *E. erythrogaster* did not expose the aposematic ventral coloration consisting of bright red or orange-red spotted with black; therefore the function of the red and black ventral coloration remains unclear.

We thank Wolf Engels, Marcos Di-Bernardo, and Andreas Schlüter for support.

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5.11 **Solé**, **Mirco**[1]; Pelz, Birgit[1] & Kwet, Axel[2]

Hyla faber (Smith frog) Diet.

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Herpetological Review (2004) 35: 159

Der Schmied (*Hyla faber*) ist eine der größten Laubfroscharten Südamerikas. Die Daten über seine Ernährung sind bislang rein anekdotisch. Während Feldarbeiten im Araukarienwald-Schutzgebiet Pró-Mata wurde der Mageninhalt von zwei Fröschen mittels Magenspülung gewonnen. In einem Fall fanden wir einen gut erhaltenen Knickzehenlaubfrosch der im Untersuchungsgebiet häufigen Art *Scinax granulatus*. Dieses Tier wurde wahrscheinlich nur kurze Zeit vorher erbeutet, da der Verdauungsprozess noch nicht begonnen hatte. Im Magen des zweiten Frosches fanden wir Überreste eines *Aplastodiscus perviridis*. Es handelt sich um den ersten Nachweis für Anurophagie für *H. faber*.

HYLA FABER (Smith Frog). **DIET.** During fieldwork on the *Araucaria* Plateau of Rio Grande do Sul, southern Brazil, various leptodactylid species were collected and stomach-flushed using the methodology described by Patto (1998. Herpetol. Rev. 29:156–157). On 27 Dec 2001 a male *Hyla faber* (95.3 mm SVL, 54.33 g), was observed within a pond near the Pró-Mata research station (29°30'S, 50°10'W) by M. Wittmann. As this specimen seemed to be unusually fat for a calling hylid male it was collected and stomach-flushed. A single adult male *Scinax granulatus* (36.2 mm SVL), one of the most common frogs of that region, was found in the stomach. The flushing of another male *H. faber* (87 mm SVL, 55.46 g), captured on 16 Nov 02 at 2300 h by BP near the Pró-Mata reserve, yielded insects and the fragments of an anuran leg (tibia and femur). On 5 Dec 2002 at 2200 h, a male *H. faber* (95.9 mm SVL, 66.36 g) caught on the border of an *Araucaria* forest near a small pool within the Pró-Mata reserve by MS contained an adult partly digested *Aplastodiscus perviridis*. Most hylids do not feed during the time they spend at the reproduction sites, but little is known about the diet of *H. faber*. To our knowledge this is the first report of anurophagy for this species.

We thank Marcos Di-Bernardo, Wolf Engels, and Wolfgang Maier for their cooperation.

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10. Lebenslauf

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