

# ZooKeys 500: traditions and innovations hand-in-hand servicing our taxonomic community

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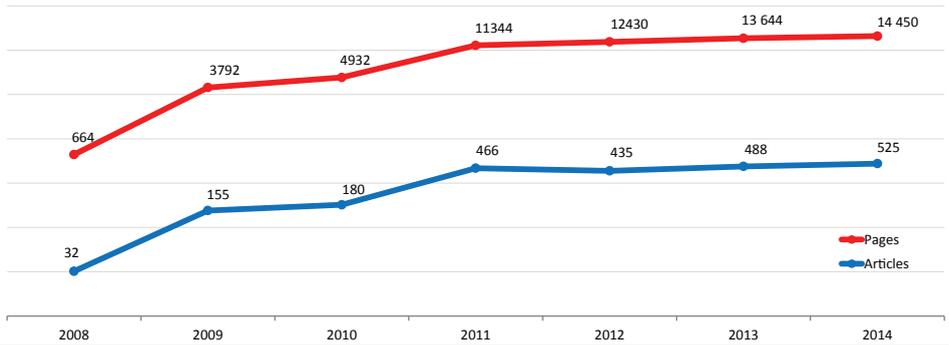
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On 27<sup>th</sup> of April 2015 ZooKeys published its jubilee issue 500. It has been exactly 28 months since we published our semiquincentennial issue (Penev et al. 2012) and made a review of the journal's progress since its establishment in 2008. Now, reaching this milestone makes us cast a look back to see what we have achieved in the passed two and 1/3 years.

## And...we have a lot to be proud of !

From its start in July 2008 through April 2015, the journal published altogether 2436 articles and 65942 pages. The number of published articles continued to grow gradually over the last two years (see Fig. 1) reaching respectively 488 in 2013 and 525 in 2014. Likewise, the number of published pages increased from 12430 in 2012 to 14450 in 2014. The total number of submissions since the launch of the journal on 4<sup>th</sup> of July 2008 reached 3407 or approximately 42 manuscripts per month, on average. Launched as a fast-line publishing journal, in spite of the great increase in submissions, the average peer-review and production time remained within the timeframes of 2012, namely approximately 3 months from submission to publication. The actual rejection rate based on evaluation of all submitted versus published articles is 28%.



**Figure 1.** Total number of published articles and pages for the period 2008–2014.

Over the last two years, ZooKeys continued to increase its role in taxonomy sustained by implementing new publication models and technologies. In a race with the rapid destruction of ecosystems on the planet, the journal is seen as the best venue for describing the world's biodiversity at a fast pace. Since 2008 until now, ZooKeys published altogether **5973 new taxa**, of which **5565 new species or subspecies**, **382 genera** and **26 families** (see also Table 1). It made its way to the top 10 journals publishing the greatest number of new taxa in Zoology reaching currently a second place in Thomson Reuters' Index of Organism names right after Zootaxa. This accounts for 5.55% of all newly described animal taxa. In terms of nomenclature proposals it also ranks second with a share of 6.15% of all published acts (according to Thomson Reuters' ION, accessed 18 April 2015). The journal is also in Zoological Record's top ten publications containing new taxa with the publications of Marsh et al. (2013) and Fernández-Triana et al. (2014) on braconid wasps from Costa Rica, ranked respectively second and ninth.

**Table 1.** New taxa published in ZooKeys compared to all described animal taxa (data from Index of Organism Names, ZooBank and Pensoft's Journal System).

Categories	Total described (all journals) 2009–2014 (ION)	Described in ZooKeys 2009–2014	% described in ZooKeys from all described 2009–2014
Species-group names	95377	5541	5.81%
Genus-group names	10204	381	3.73%
Family-group names	1501	26	1.73%
<b>Total</b>	<b>107082</b>	<b>5948</b>	<b>5.55%</b>

A number of technological and administrative measures were undertaken in the last two years to advance even more our journal's position in the global publishing market. These were not left unnoticed by the scholarly community, data registries and leading indexers of scholarly literature. The ZooKeys impact factor, as evaluated by Thomson Reuters' Journal Citation Reports for 2014, continue to grow and from

0.514 in 2010 reached 0.917 in 2013. Likewise, SCOPUS SJR indicator shows increase from 0.26 in 2010 to 0.48 in 2013.

In February 2013, Pensoft announced the integration of all its journals with CLOCKSS [Controlled LOCKSS (for Lots of Copies Keep Stuff Safe)] archive, which guaranteed their long-term preservation, integrity and perpetuity. As an added benefit, Pensoft became a partner of the Global LOCKSS Network supporting libraries and their local collections.

Being the first taxonomic journal to promote and implement data publishing in its routine workflow (Chavan and Penev 2011), over the last two years ZooKeys strengthened its leading position in this field and was recognised as the most reliable venue for publication, integration and dissemination of taxonomic data. From November 2011 when the first data paper was published in the journal (Narwade et al. 2011) until now, their number increased to 37 covering various aspects of biodiversity knowledge.

A major step towards strengthening the journal position was undertaken in December 2014 when ZooKeys moved on to a new technologically advanced publishing platform with several innovative features that better visualise published content and maximizes its re-use by readers. These include a navigation panel that allows key text elements, such as figures and tables to be downloaded individually. Other key features include visualisation of occurrence data on interactive Google map, Taxon Name Profile, and Reference finder (<http://refindit.org>). Besides, a new article level metrics allowing scoring the number of user's visits by article format, as well as the number of views of each individual figure and table was introduced.

In November 2013, with the publication of issue 346 ZooKeys initiated an automated registration of new taxa with ZooBank. This was achieved through a server-to-server communication from the journal to ZooBank and back, using the TaxPub schema, which is an extension to the Journal Tag Publishing Suite (JATS) of the National Library of Medicine (NLM) (Catapano 2010, Penev et al. 2011). By doing this, ZooKeys became the first journal ever to implement such work flow in its publishing system and one of the first taxonomic journals accepted for archiving in PubMedCentral. Next to come is pipelining registration and publication of other types of nomenclatural acts.

The last two years will also be recalled with the publication of several landmark thematic monographs and conference proceedings, just to mention a few: Contributions to the systematics of New World macro-moths IV (Schmidt and Lafontaine 2013 – ZooKeys 264) and V (Schmidt and Lafontaine 2013 – ZooKeys 421); Advances in Hemipterology (Popov et al. 2013 – ZooKeys 319); DNA barcoding: a practical tool for fundamental and applied biodiversity research (Nagy et al. 2013 – ZooKeys 365); Review of taxonomy, geographic distribution, and paleoenvironments of Azhdarchidae (Pterosauria) (Averianov 2014 – ZooKeys 432); Proceedings of the Summer Meeting of the Crustacean Society and the Latin American Association of Carcinology, Costa Rica (Wehrtmann and Bauer 2014 – ZooKeys 457); The origin and early evolution of metatherian mammals: the Cretaceous record (Williamson et al. 2014 – ZooKeys 465).

Quite a number of interesting zoological discoveries were announced in the journal and attracted large audiences and considerable media interest (see also Tables 2 and 3). Among those, worth mentioning: a new procyonid mammal, the Olinguito, from the Andes (Helgen et al. 2013); a new genus of monk seals from the Caribbean Sea (Scheel et al. 2014); a new genus and several new species of bats from Africa and the Neotropics (Reeder et al. 2013; Velazco and Patterson 2014); a new subgenus and four new species of electric fishes from the Amazon and Congo river basins (Sullivan et al. 2013; Lavoué and Sullivan 2014); a new genus and species of rove beetles collected by Charles Darwin 180 years ago and published on his birthday (Chatzimanolis 2014); a new genus and species of ancient clams found in the depths of the Arctic Ocean (Valentich-Scott et al. 2014) and many others.

Shortly after the erection of the method of rapid and *en masse* descriptions of new taxa, often called “turbo-taxonomy” in 2012, ZooKeys served as an experimental testbed for the concept (Riedel et al. 2013, 2014). Furthermore, entirely new methodological approaches in taxonomy were introduced in the journal, among others a new LEGO pinned insect manipulator (IMp) (Dupont et al. 2015); a new illustration technique allowing integration of scanning electron microscope images into an interactive rotatable model (rSEM) (Akkari et al. 2013); a new set-up for production of highly detailed quality pictures of pinned insects (Brecko et al. 2014).

**Table 2.** Top 10 most accessed press releases of ZooKeys articles posted through EurekAlert! (from the EurekAlert! counter) for the period 1 December 2011–13 April 2015. The counter registers only downloads from EurekAlert! mostly by science media and journalists. The actual number of readers is actually much higher than this number.

Title	Author/s and year of publication of the original article	Date posted	Press release views on EurekAlert! website
<i>Megalara garuda</i> : the King of Wasps: A new, giant wasp comes from Indonesia	Kimsey and Ohl 2012	23-Mar-2012	44 669
World's smallest frogs discovered in New Guinea	Kraus 2011	12-Dec-2011	44 247
Spider version of Bigfoot emerges from caves in the Pacific Northwest	Griswold et al. 2012	17-Aug-2012	16 361
Your small-living-creature shoots may benefit big science	Goula et al. 2013	30-Jul-2013	12 640
A new trout species described from the Alakir Stream in Antalya, Turkey	Turan et al. 2014	12-Dec-2014	9 602
Striped like a badger – new genus of bat identified in South Sudan	Reeder et al. 2013	9-Apr-2013	8 402
New scorpion discovery near metropolitan Tucson, Arizona	Ayrey and Webber 2013	19-Feb-2013	6 631
A new species of moth from the Appalachian Mountains named to honor the Cherokee Nation	Quinter and Sullivan 2014	25-Jun-2014/	6 466
Ninety-eight new beetle species discovered in Indonesia	Riedel et al. 2014	16-Dec-2014/	6 400
Mummy-making wasps discovered in Ecuador	Shimbori and Shaw 2014	8-May-2014	5 423

**Table 3.** Top ten most viewed articles of ZooKeys by unique views (according to the ZooKeys website counter accessed on 21 April 2015).

Article	Views
Helgen et al. 2013 – Taxonomic revision of the olingos ( <i>Bassaricyon</i> ), with description of a new species, the Olinguito	49 471
Griswold et al. 2012 – An extraordinary new family of spiders from caves in the Pacific Northwest (Araneae, Trogloraptoridae, new family)	48 181
Bouchard et al. 2011 – Family-Group names in Coleoptera (Insecta)	27 435
Hagedorn et al. 2012 – Creative Commons licenses and the non-commercial condition: Implications for the re-use of biodiversity information	24 237
Sereno 2012 – Taxonomy, morphology, masticatory function and phylogeny of heterodontosaurid dinosaurs	23 846
Winterton et al. 2012 – A charismatic new species of green lacewing discovered in Malaysia (Neuroptera, Chrysopidae): the confluence of citizen scientist, online image database and cybertaxonomy	22 629
Kraus 2011 – At the lower size limit for tetrapods, two new species of the miniaturized frog genus <i>Paedophryne</i> (Anura, Microhylidae)	17 054
Penev et al. 2009 – Data publication and dissemination of interactive keys under the open access model	16 988
Sereno and Larsson 2009 – Cretaceous Crocodyliforms from the Sahara	16 966
Cerretti et al. 2013 – A neotype designation for the bone-skipper <i>Centrophlebomyia anthropophaga</i> (Diptera, Piophilidae, Thyreophorina), with a review of the Palaearctic species of <i>Centrophlebomyia</i>	16 938
ICZN 2012 – Amendment of Articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication	15 578
<b>Total</b>	<b>279 323</b>

The journal success wouldn't be possible without the great support of the zoological community. We deeply appreciate the help received from our most active authors, reviewers and editors!

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# Terry L. Erwin: She Had a Black Eye and in Her Arm She Held a Skunk

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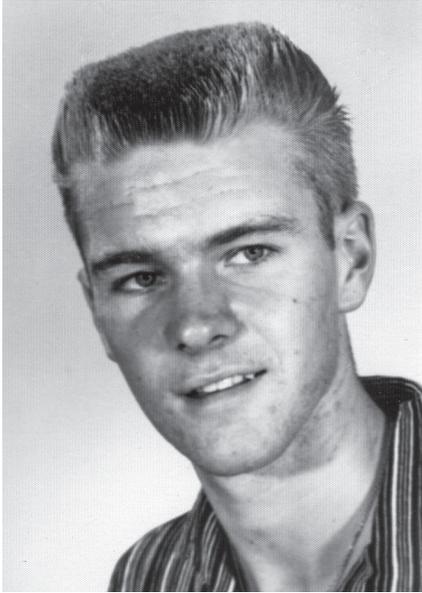
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Terry L. Erwin is Curator of Coleoptera at the Smithsonian Institution, National Museum of Natural History, in Washington, D.C. and editor-in-chief of *ZooKeys*. He generated significant controversy in 1982—which continues to this day—when he published an estimate of 30 million species on Earth, which was substantially more than the nearly one million described species. He was born 1 December 1940 in St. Helena, California and spent his youth trout fishing with his maternal grandfather in the High Sierra near Lake Tahoe. As a teenager, with prodding from his father, he built hot rod cars and was a founding member and later President of the California Conquistadores, a hot rod club in the San Francisco Bay area. Erwin earned his B.S. (1964, Biology) and M.A. (1966, Biology) degrees from San Jose State College. With a desire to learn from the three greatest living carabidologists, he first obtained a Ph.D. (1969, Entomology) from University of Alberta under the direction of George Ball. This was followed by a post-doctoral fellowship at Harvard University's Museum of Comparative Zoology



Terry Erwin, senior, Vallejo Senior High School, 1958.

with Philip J. Darlington, Jr. During that year, a position opened at the (then) United States National Museum in the Department of Entomology, which he accepted, but two months after taking the job, he departed for a year-long sabbatical at Lund University in Sweden, where he completed the carabidology “trifecta” under the mentorship of Carl H. Lindroth. While in Sweden, the Chairman of Entomology at the USNM changed from Karl Krombien to Paul D. Hurd, who saw on his desk a proposal left by Terry to study California carabid beetles. Learning that grant money was available for research in Central America, Hurd crossed out “California” and wrote in “Panama.” Terry returned to Washington in 1971, as the second coleopterist within the USNM, and was greatly surprised to find that his proposal had been changed, funding had been secured, and he was scheduled for

the next flight to the Canal Zone. Thus began a lifetime career on studies of insect biodiversity in neotropical forests.

This interview began in Austin, Texas on 13 November 2013 with The Macallan 18 (a single malt scotch) and a toast “to all things on six legs.” It concluded in Portland, Oregon on 17 November 2014; Erwin was two weeks short of 74 years old.

*Rice: What I want to do, Terry, is interview you for a new column in American Entomologist called Legends. And I’ll do this column for five years, or until I run out of energy.*

Erwin: [*Laughs.*] This column is a good idea; an excellent idea for ESA.

*You are still active in biodiversity and conservation, but I really wanted to narrow this down and look at the entomological aspects and to communicate to entomologists broadly, so some of these questions will be elementary, but some will be philosophical and you just run with it any way you want.*

Okay.

*Who is the person, or what was the event, that motivated you to study entomology?*

Those are always great questions, and I know there are just all kinds of diverse answers you get from everybody, but probably mine is kind of like a common one, and that is J. Gordon Edwards. He was a professor at San Jose State College; it was college then and not university, ’cause this was back in the 60s. My father was a race driver—a tin knocker—and he didn’t finish high school, and when he retired from Mare Island Naval Shipyard, he was a nuclear engineer building atomic submarines, and that was my path. My grandfather worked at Mare Island, my father worked at Mare Island,

my mother, my uncle. Vallejo was a very small town, so that was it. You grew up and worked at Mare Island. I actually did four summers there to help pay for my college.

*You built atomic submarines to pay for college?*

I was working in the atomic reactor room of the Polaris missile [USS] *George Washington* submarine. My job was to carry buckets of asbestos mud, so they put it on the preformed stuff, then they would wrap it with a fiberglass cloth. Anyhow, I was waiting for my call. I was just sitting there in the lower level and I leaned up and it was all wet; on my arm was chewing tobacco where somebody above had gone "pitooyey." I looked at that and wiped it off and said this is not for me. [*Laughs.*] So, that was it. I was in junior college taking electives and because I had read a book by James Michener about Hawaii and the Polynesians and their teeth problems, I decided to become a dentist. So I took zoology and thought it was pretty cool. Then I was in a discussion with somebody and they said, "Do you want to spend your life like this?" [*Mimics a dentist staring into a mouth.*]

*Leaning over, putting a drill into somebody's mouth!*

Exactly. I just realized I really didn't want to do that, so I went to San Jose State. I had a favorite English professor in junior college, so I minored in English and majored in life science teaching. During that time, I had to take two life science classes, one of which was marine biology with Polly McMasters and the other was entomology from Gordon Edwards. Polly would get her class up at four o'clock in the morning and go over to Moss Landing and dig up polychaete worms. Frozen fingers and just...gawd! Then we would go back to San Jose and in the afternoon, Gordon Edwards would get out the butterfly nets, and we would go out to Allen Rock Park and collect insects in the warm sunshine. Didn't take me long to figure out what I wanted to do. [*Laughs.*]

*Definitely not a marine biologist?*

Definitely not. And also Gordon was just a really dynamic personality, just fantastic. He recruited maybe seven or eight students per year. I just switched to entomology and the interesting thing during that phase was my English classes were dragging me down. I was on probation with a D average and I aced Entomology 51. That was back in the "Pleistocene" when it was 51, not 101. Then I got A's the entire rest of my student career. It was because of Gordon and his professionalism as a professor and the fact that [when] you brought your insect collection and if there was a *Musca domestica* there, he would just *salivate*, "how great that's pinned; that's really a great specimen!" A student just jumps on all that kind of feedback. And that was it.

*It is usually one individual and it was Gordon Edwards for you.*

He was the one.

*Being an "A" student, did you have any challenges during graduate school?*

No, actually everybody wanted me to go to Berkeley. There were some great coleopterists there like E. Gorton Linsley. But I said as soon as I walk on campus, I'm go-



Terry Erwin, Curator of Coleoptera, Smithsonian Institution, 2004.

ing to be the carabid expert, so I've got to go somewhere else. I wrote to Carl Lindroth in Sweden, who had just published his volume three of the carabids of Canada and Alaska. So I wrote to him and he said, "Well, you are already working on bombardier beetles, and if you want to do that for your Ph.D., I'm not the right person. You really should go to George Ball." George who? [*Laughs.*] I wrote George and got back a letter; he was on an 18-month sabbatical in Mexico collecting carabids. He said, "Okay, I'm going to be down here for a little while, but why don't you just drive on up [to Edmonton, Alberta] and find a place to live and I can support you the first year with pinning my Mexican carabids." He said just check in with Brian Hocking, the Chair of the department. So my [ex-]wife and I arrived at the Hocking house, and Jocelyn, the wife, opened the door, and she had a black eye and in her arm she held a pet skunk. [*Laughs.*] She was this little British woman with a very nice accent who, unfortunately, connected with a badminton birdie in her eye! "Welcome. They said you were coming." And so they helped us through the first week and we got a place to live. George supported me for the first year; then I got a Queen Elizabeth Scholarship for the next two years. I finished it in three years.

*What was the Queen Elizabeth Scholarship? Was that a full ride?*

Yeah, a full-ride scholarship of \$2,600 a year. [*Laughs.*] It did fine and that's actually a Canadian grant. The idea was to finish off as soon as possible. Then Phil Darlington gave me a post-doc and I went from Edmonton to MCZ [Museum of Comparative Zoology]. Then Oscar Cartwright, the old coleopterist at the Smithsonian, retired and they asked George Ball, who was visiting there, "Can you recommend anybody?" He said, "Well, yeah, I just had a student graduate. He's at Harvard right now, and I'd recommend him." So they called me and I said, "No, I don't want to come. I want to do a post-doc with Carl Lindroth in Sweden." They said, "You can do that too, so come on down." That's when I had my first sabbatical. I was in Washington for two months; then I went to Sweden for a year. After that, I had worked with three of the top carabidologists in the world, and that really was my objective.

*Let's jump forward and look at your career. What do you consider your most significant contribution to the field of entomology?*

I think this one-page *Coleopterists Bulletin* paper, for one thing, started a cottage industry in fogging, so that became a real technique to look at the forest canopy, and the second thing was a cottage industry in shooting me down [*laughs*] from my naïve hypothesis built on some naïve assumptions, and naïve arithmetic, and coming up with the 30 million [species estimate]. But the point is that most people never realized, that wasn't the point of the paper. That was a throwaway last paragraph. The point of the paper was that Peter Raven [then Director, Missouri Botanical Garden] called me and he was doing something with the National Research Council, where they needed to know how many species were in an acre of Panama. That was the question. And I said, "Peter, nobody knows that stuff about insects. It's just impossible." I had done Panama fogging in the tree *Luhea semannii*, and I said, "Well, give me some time and let me see



Terry Erwin fogging for insects in the Amazon Basin, 2014.

what I can do.” And so I went through and analyzed all that stuff with those numbers and I came up with 46,000 species per hectare in Panama. He took that and that was great; so let me put this in a little paper for *Coleopterists Bulletin*. Well, if we know this for one tree, how many trees are there in the world? Fifty thousand? Okay, how many insects are host specific? Who knows, but try 13 percent, and so that came to the 30 million. Several people came and said, “Well, what if it’s five percent? What if it’s 20 percent?” And so forth. Those numbers have been batted around and they’re still batted around. The really interesting thing was that Yves Basset, from STRI [Smithsonian Tropical Research Institute], just published a paper last January in *Science*, where he had 110 taxonomists and 10 years of collecting with several different kinds of methods. He came up with a minimum of 28,000 and a maximum of 44,000, based on all of that. And I did it on one tree and some simple math and came up with 46,000! Actually, that’s probably pretty close. We now know that there are probably over 100,000 [species] per hectare in the western Amazon Basin.

*I checked on the paper in The Coleopterists Bulletin; it has been cited, according to Google Scholar, 835 times.*

Yeah, I think it just hit 848.<sup>1</sup>

<sup>1</sup> 920 citations as of 1 January 2015.

As long as we are on this number of species, you had estimated 100,000 species per hectare based upon your work in western Ecuador. You have also mentioned 17 billion hectares in the Amazon Basin. Did you provide a number for species?

No. My usual throw-away line is 100,000 species per hectare and  $3.2^{10}$  individuals per hectare in the western Amazon Basin. There are 17 billion hectares and 450 different kinds of forest. Do the math. So that's my line—do the math.

*You are not going to lay a number out there and be quoted?*

Right. No. [*Laughs.*] My point this morning [during the symposium] was that the way we collected those things using the garden hose to wash them off the [fogging] sheets, that's the sample I used to get there. So what if I missed 50 percent of the specimens because they got washed away or...then the 30 million would have been higher.

*You have had a tremendous career studying carabids, but why study beetles, and especially beetles that inhabit the rain forest canopy? What got you into the rain forest high up in a tree?*

Why don't we just step back to beetles? Gordon Edwards was the coleopterist and he had a very nice collection and he encouraged us. I started with cerambycids with three of my buddies, who are all cerambycidologists and they were *very* competitive; I mean, *really* competitive. So I asked Gordon if I could have another family. He said, "Well, Carl Lindroth had just published volume three on *Bembidion* and I have been collecting at Glacier National Park and the Tetons and I have lots; you could key those out." So, that's how I got into carabids. Before I left for Sweden, Karl Krombein was the Chair [of the department] and he said, "Leave me some proposal about what you are going to do when you come back." So I wrote a little proposal to do the carabids of California, because I had a hundred thousand [specimens] that I had collected as a student. When I came back, Paul Hurd had taken over as chair and he found out that there was some money to work in Panama, and so he got my proposal—he crossed out California and wrote in Panama. So I ended up, for seven years, working and going back and forth to STRI on Barro Colorado [Island] and that is how I got involved in the fogging. As I said, when those things came down and I saw these rare carabid beetles on the sheet, I said *that's* how I have got to collect the carabids of the canopy. But it sort of went out of the box from carabids into biodiversity because of the 30-million paper and then that went into conservation and so forth, and the box just kept getting bigger because of that first fogging event.

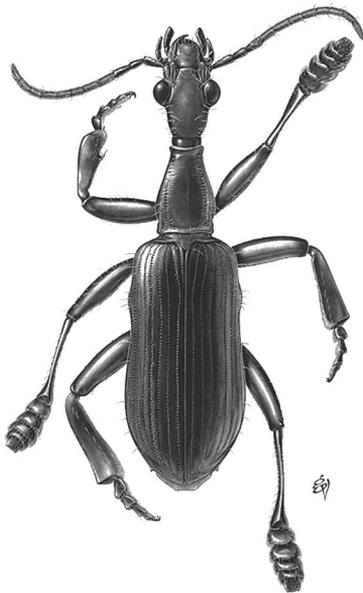
*It was like opening a Christmas package.*

Oh man, it's just unbelievable. And the genus that I've been working on for a number of years, which is the genus *Agra* [and] which is strictly canopy—it has turned out to be a lot of fun naming things in the genus *Agra*, but there are just over 500 species described, many of which by me, and in the museum from all my borrows and all my foggings, I have over 2,000 species. So that means I've got like 1,600 species that need names. And you know, it's the last biotic frontier. Until we started fogging,



*Agra dax* Erwin  
Illustration by Marlin E. Rice

*Agra dax*.



*Agra sasquatch*.

her; unfortunately, he died first. But anyhow, he told me her nickname is Cat. So *catbellae* is the species name and I turned that into “the belly of the jaguar” and that was in relationship to the demise of the jaguars’ rainforest.

nobody knew what was up there. Now we know the average size of a canopy beetle is 3 millimeters. So you think of architecture: well, the finer you get, the smaller the twigs, the smaller the insects.

*Two thousand species of Agra! That has been the focus of your research. I pulled three names off the web: Agra cadabra, Agra vation, and Agra katewinsletae. Give me some context to those names.*

*Agra vation* you could guess right away. *Agra cadabra* is a play on words. *Agra dable*—my [current] wife is Peruvian—and this was a very pleasing, nice species. So we speak Spanish and “*agradable*” means very pleasing. [Laughs.] *Agra katewinsletae* for Kate Winslet, *Agra liv* for Liv Tyler, *Agra catbellae*, which is Catherine Bell, so all of my heart-beating [*pats his heart and sighs*] female movie stars can get a name if they star in a movie where there is a disaster. Okay, so the *Titanic* goes down; in my etymology, the analogy is the destruction of the rainforest and the *Titanic* going down.

*Okay.*

Liv Tyler was in *Armageddon*, so they are the same thing; the destruction of Earth, the destruction of the rainforest. So all of those celebrity names have to have something to do with disasters. Catherine Bell is a star of *JAG*, a lawyer—she is just luscious. But her nickname was Cat. Did you know Frank Hovore?

*Yes, I knew Frank.*

Well, Frank used to hit golf balls at the driving range with Cat Bell. He always promised that he would introduce me to her; unfortunately, he died first. But anyhow, he told me her nickname is Cat. So *catbellae* is the species name and I turned that into “the belly of the jaguar” and that was in relationship to the demise of the jaguars’ rainforest.

*That's clever. You have given the names some thought.*

Yeah, all of those. We did a Smithsonian Channel hour, two years ago. My post-doc, C. J. Gerasi, and I went into the studio, sort of like a Jay Leno kind of set up, and Susan Spencer is the interviewer. So I come in first and we are chatting a little bit, then C. J. came in and we sat there for a little while and Susan says, "I understand you've named some species after movie stars. Did you ever name one after a man?" I said, "Well, of course, after my professor and people who have collected." "Well, any movie stars?" I said, "Yeah, I did and I had this one species of *Agra* that had this middle femur; big, big femora, so I named it after Arnold Schwarzenegger—*Agra schwarzeneggeri*. That was the one man, and then he became the governor of California." [*Laughs.*] Then she said, "Oh, yes." She pulled out this beautiful blue folder with gold lettering from the Office of the Governor of California. My students had done an image of *schwarzeneggeri* and sent it out to him and he signed it, "Thanks for thinking of me—Arnold." [*Laughs.*] Anyhow, that is the only one I've named after a male star and its physical attributes had nothing to do with movies.

*What is your passion in entomology—the thing that most motivates you or brings you the greatest joy?*

Curating the national collection. I'm the only coleopterist on the Smithsonian side. I have four USDA colleagues in Coleoptera and each one of them is a contact person for their family. Sasha Konstantinov has chrysomelids, Steve Lingafelter has cerambycids, Lourdes Chamorro is our new curator of weevils, and then Nat Vandenberg is the identifier person. So I have all 165 *other* families in my responsibility and thanks to David Furth, many of those have now been deactivated. So they and my research assistant, Charyn Micheli, and the collection manager for Coleoptera are in charge of 12 million specimens, and of course, nobody can handle that, but then when you bump it down to my responsibility with carabids, we have a little bit over one million carabid beetles. So my goal in my career is to leave that collection just immaculate; as many identified as possible, new species identified as new species, but maybe not described, but everything sorted, everything in perfect order and it is great therapy—just to go in and curate drawers of Coleoptera. Of course, I actually start with the groups I am actively revising and get those done, but then I'm doing a series of books now—the *Carabidae of the Western Hemisphere*. It's going to be 10 volumes, three are published, the fourth is almost done, I'm starting on five, and there's 40,000 species of carabids described; just over 10,000 from the Western Hemisphere. So the idea is that's one legacy project I'm working on, is the ten volumes. To support that is the other legacy, which is to get the collection in perfect shape. That is what I enjoy most.

*Describe the experience; when people hear the word Smithsonian, something majestic comes to mind, and for somebody to work there, it's probably like working in a royal palace.*

It is, except the clothes of the royalty are tattered, hand-me-down pants and shoes, [*laughs*] and it's absolutely awful. My departmental budget, annually for each curator throughout the seven departments, is \$2,000. That's all we get: \$2,000. That \$2,000

brings me to the ESA meeting every year, and if I want forceps, I have to buy them out of my own pocket. They give us a phone—no charge—and every three years we get an updated computer system—Dell—and updated is not quite correct. What we have to do is take our old ones, turn them back to Dell, then they give us last year's model. Anyhow, royalty is all a façade, but just the fact is that we have the greatest, accessible collections in the world. Paris [Musée National d'Histoire Naturelle] probably has more specimens, but really not very accessible. You have to go into the attic and look in old boxes and stuff like that. So in that sense, it [the Smithsonian] is a great place to work. The downside is that we can't go to NSF [National Science Foundation] for funding. I've never been able to actually get nice big bunches of money where I could do a five-year project and expect to do it each year, and I just have to beg and borrow year after year after year to do anything. So that part of the Smithsonian really sucks.

*Everyone has a story to tell. What is a favorite memory of your career?*

In 1976, I think, was the International Congress [of Entomology] in Washington. I decided to do the first international symposium on carabidology and set it up for three days; a symposium with lots of talks. All the carabidologists came; Darlington and Lindroth came over, and I used the State Department to bring some of our folks from behind the Berlin Wall; Fritz Hieke from the Humboldt [University]. In those days, it was really difficult to get those people out [of East Germany]. We had more than a hundred people interested in carabidology and David Maddison was our youngest at 17. Phil Darlington's talk was about standing on the shoulders of giants. It was just really a dynamic time. I was living in an apartment at that time and Dave Kavanaugh and a couple of my colleagues from Europe were sleeping on the living room floor. You know, it was just really an excellent time. I mean, we've had a lot of good times after that, but I think that was a special time.

*Back to the Amazon. What do you hope will be the outcome or the long-term impact of your research?*

That's a good question. I'm hoping that as we get the rest of the 2005-2006 and the current samples from this year, get that all in so that we have a 20-year image of what's going on, then I can tie down these numbers, like the 100,000 species per hectare in *Science* or *Nature* or something like that. That will wake people up to the fact that, yeah, we might have 30 or 50 million species or a lot more on the planet, but we are knocking them off a million at a time. So I think just awareness.

*Give me a perspective. When you fog, how much diversity or numbers of things do you find in a year, or how much have you collected in total over all of your efforts over all the years?*

Okay. In fogging, the important thing to do is to ask the question and then design the experiment using the fogging system to answer that question. So that may mean you climb a tree and fog just the canopy of that particular tree, or in this recent thing where we are doing bio-monitoring of the oil company road, we wanted actually a picture of the entire forest and see what the impact of the road building and the use of the road by

oil trucks is, and so that stretched over twenty years. We just finished up last year; three intense years to start when they were building the road, then the 10-year follow up and the 20-year follow up. We have about nine million specimens from twenty-four hundred samples. Each sample, when you fog standing on the ground up into the canopy, each sample has an average of about 2,800 specimens on a sheet that is three meters by three meters.

*Wow! Nine million specimens.*

We have 100 sheets for each seasonal visit and we have no idea how many species on that particular sheet, but now as a result of 20 years of looking at everything, doing some extrapolation looking at some taxa, we know how many species there are and the relationship of that taxon with all the published ones. We suspect now that there's over a hundred thousand species in one hectare of equatorial rainforest in the Western Hemisphere; a hundred thousand species of insects and their relatives and the real Carl Sagan number, the individuals in that hectare, [is] 3.2 times 10 to the 10th individuals. So that figure has no name; you just have to say 10 to the 10th and that's what we're getting in a long-term study. If you just go out and fog one tree (one tropical tree, for example), you get about seventeen hundred species; depends on the tree, of course—the next tree might have three thousand species. It just depends on the toxicity of the tree and all kinds of questions like that, but the main thing is, for this oil company road, the rule was that the road could only be 27 meters wide because of various problems Ecuador had with previous oil companies. They put rules and the virgin rainforest had to be intact on both sides of the road, so for insects, after 20 years, there was little or no impact on the entomofauna from that road. However, all the bushmeat was gone in three years. I started with five species of monkeys in the plot; at the end of three years there were no monkeys, no tapirs, no cats, no crassid birds—currasows—anything that was edible was gone.

*How far away from the road was this megafauna depleted?*

This is the territory of the Huaorani indigenous folks, and before the road, there were 70 dispersed families across two million hectares. [Here's] a picture of the Huaorani: they have big wooden disks in their earlobes, some of them file their teeth, they don't have very many clothes, they have blowguns, and they go off for days trying to hit a monkey with a dart. Once the road came in, they dressed in western clothes, they had rifles and they knew how to hitchhike on oil company trucks and this road is 121 kilometers long, so driving back and forth every day hunting, they wiped out the megafauna, or the bushmeat, as we call it, for one to two kilometers back from the road on both sides. The good thing was, 10 years later, most of those old hunters were a little too decrepit to go hunting, and the teenagers—I actually had two teenagers, Huaorani, helping me on the project—they didn't remember or were never trained on how to follow an old machete trail. So ten years in, my plot had grown in and I asked them to go and clear out this thousand-meter trail to the back of my transect. I was teaching my students how to tie knots and hang up sheets and stuff like that. After half an hour I followed the two [Huaorani] guys and the trail was curving. What's going on here? I finally caught up with them and they had no idea how to follow scars on the

little bushes that were cut ten years before [by machete]. And so, I had two monkey species back in my plot; so there's hope.

*So the monkeys are moving back into the plots.*  
Yeah.

*I want to take you back to the nine million specimens you collected. What is one of the most unusual things, dramatic things, exciting things that you caught—insect-wise—in your nearly 40 years of fogging in the Amazon?*

One of the very interesting things about these 2,800 specimens that come down, on average per sheet, is once you start parsing out the individuals and looking at the same sheet through the dry season, rainy season, and transition season, which is what we did for each time that we went down, 51 percent of the catch across all 2,400 samples—51 percent were ants. So the majority of abundance is ants no matter where you go. That's amazing, absolutely amazing. But the next thing that is really, really interesting is you get walking sticks and praying mantids of such camouflage that you just can't image how these things evolved to blend in with their tree trunks and the leaves and lichens; it's just amazing. But for me, the most very interesting thing, and I tell this to the hymenopterist at [University of California] Riverside—Heraty, John Heraty. I've admitted this to John Heraty and I hate to put it in print, but if the micro-hymenopterists would get off their lazy asses and start describing species, there would be more micro-Hymenoptera than there are Coleoptera.

*Really! You think so?*

Absolutely, because every beetle, every weevil, has a parasite and those little tiny micro-hym parasites have hyper-parasites of littler micro-hyms. I mean, it's a no-brainer. But what I wanted to say about that is, when you look under the scope at this tremendous biodiversity that's in the canopy, the *neatest* thing architecturally are the micro-hyms—they're just unbelievably fantastic. And don't tell John, but if I had to do it over again, I might have been a hymenopterist. [*Laughs.*]

*Well, it's unfortunate that the entomological community can't see this diversity that you are talking about to learn to appreciate what's out there.*

That's the real thing, when you actually get one of these canopy samples and get little spoonfuls in a little plate under the microscope to see the incredible diversity of forms and species and all that kind of stuff that's in the canopy, that's actually when you appreciate how much biodiversity's out there and this hundred thousand species per hectare. Now that's the Western Hemisphere; the Amazon Basin has 17 billion hectares, and in those 17 billion hectares there's 450 different types of forest, and each of those forests have *subtypes* of forest within them, so my 30 million estimate is so conservative that it's just hard to imagine what's really out there.

*So, what's your new estimate?*

It's impossible to say, absolutely impossible.

*I can't get you to give me a number, can I?*

No, no! [Laughs.] It's just impossible to say, but the thing is, I'm getting them on the hoof and we're looking at morphospecies, but then the gel jocks are going into a species—quote unquote—and finding out that actually that maybe there's five or six molecular species within that taxonomic species. And so that makes even my samples more diverse than just what you can see with your eyes, and so then, that gives me pause to make another estimate, because they are just getting started with how many siblings are in a morphospecies. So, no—impossible.

*You spent time in the Amazon Basin over a period of several decades. Did you ever encounter a dangerous or threatening situation?*

The first time I was in the Amazon was 1977, so that's probably 30-plus something years. [Laughs.] It seems like longer than that. You know, I've seen snakes and all that kind of stuff and been stung by *Paraponera*.

*Really? Let's stop there. Describe being stung by the bullet ant.*

It's a *real* shock when you get stung and you know *immediately* what it was. The first time was on the back of my arm.

*You've been stung more than once?*

Yeah. So I grabbed that thing and pulled it out, and I forget who was with me, and they looked all over and there was another one on my leg and they flipped that off, so I didn't have any problems. I was just squeezing and squeezing, and then it dropped out, but they are so hard I didn't kill it and it was crawling away. That lasted for about half an hour, and by day two, oh, then after the fire, it goes to a feeling like a dull toothache and the toothache kind of goes for a couple of days and then it's gone.

*You mentioned a fire. Do you mean that the sting felt like you had been burned?*

Right. It's a severe burning sensation. The second time was in this oil company transect on the road and the oil company film team had come out and they were doing interviews in the forest and they wanted me to stand over there. I was just standing and not paying attention, but [I] was next to a *Paraponera* nest at the base of a tree and one crawled up, went out on my arm while I am giving the interview, and it stung me in the thumb. Being stung on any of the fingers is the worst thing possible.

*Because why?*

I think we have more nerve endings in our fingertips. It's a nerve agent, what they are actually putting in there. That film has more four-letter words than I [laughs] probably even I know in my conscious. I jumped up and ran in circles and they were filming me and wondering what the hell I'm doing, and I'm cussing and swearing and shaking my hand. Of course I knew exactly what it was because I had a previous experience. So those are two of my *Paraponera* experiences.

*What is the most dangerous thing in the rain forest?*

The most dangerous thing is actually a tree fall, or a branch fall. A good-sized branch comes down pretty fast and if you're under it and get hit on the head—you're dead. That's it. A tree fall, it takes a while, and you hear the crack, you look and if it's coming toward you, you just step one meter [aside] and it misses you. If you *run*, you don't know where it is coming down and it could just clobber you. It's the branches that come down that are more dangerous. One time in Tambopata [Peru] I was taking down my pulleys for pulling up the fogger, and I was pulling it out and a branch broke about that big [*makes a circle with his hands the size of a baseball*], and when they break, they kind of have a pointy thing on them and it came down and went through the hair here [*points to his forehead*], didn't hit my nose, but the branch went down, ripped my chest clothes a little bit and then ripped the material in my crotch and stuck in the ground between my legs. It was a long branch, so I'm sort of looking through the foliage and all my colleagues are standing around kind of laughing a little bit until they actually realize what happened. If it had hit me in the head, I'd be dead. When I got back [to camp], I noticed my underwear also was ripped right out, but nothing on my body. No scratches. It was so close it just took out my clothes. So that is the only time in 40 years that I have been doing fieldwork in the rain forest that anything *close* to being a disaster happened.

*Do you have a favorite insect species? It has to be a carabid.*

Oh, absolutely. My license plate says AGRA DAX, and Dax is from *Star Trek: Deep Space Nine*, and the actress, Terry Farrell—beautiful woman, absolutely beautiful. She played Dax, and actually it was Terry Farrell's body, but Dax is actually an alien parasite that lives in her, but the alien was so ugly that it had to have a different body, and what a body! Anyhow, *Agra dax* is my favorite. It's actually a very large *Agra* with a heavy body from Panama and metallic green with a rufous head with black antennal segments, so it's quite colorful, and this particular group has flattened tibiae and femorae, which means it probably lives with ants, but we don't know too much about it. Its sister species are *Agra sasquatch* and *Agra yeti*. Why? Because they have these *really* expanded tarsal segments, so then it's like Bigfoot.

*What do you consider to be your legacy, or how do you want others to remember you?*

I guess maybe by what my students do. If I've influenced my students in a good way and they go on to do stuff, then the unbroken chain just keeps going. So that's George Ball; he had 40 Ph.D. students, not just in carabids, but in other taxa, as well, and many of us have gone on like Dave Kavanaugh—my best friend and [previous] Chair and Science Director at the California Academy of Sciences. So you go back to George and to Cornell, you have Forbes, and you go back from him to Cuvier and Buffon, so there's this chain all the way from the great old-timers down through George and his students and what I'd like to do is to keep that chain going with my current student, Laura Zamorano from Colombia, and others.

*I hope I have that much energy when I reach your age.*

I've now lived in the Amazon for 16 years of my life with the various expeditions all put together, so for 16 years I breathed absolutely pure oxygen. [*Laughs.*] So that's a plus. And beetles are my hobby, as well as profession. I *never* have any stress. If there's something not quite going right, I go curate a drawer of beetles, you know. My current wife, Grace Servat, is Peruvian and is quite a bit younger than me and she kicks my ass if I'm lying around, or something. [*Laughs.*] She's an avian ecologist that specializes in the high Andes. So she's up at 4,500 to 5,000 meters in her cushion-plant zone at the very top breathing more than pure oxygen. I've been with her a couple of times when there is no oxygen for my lowland Amazon lungs, so now I just have her show me pictures and tell me about it. She does the same for my lowlands; she hates it down where there's biting bugs and [it's] hot and sweaty. So we do our own research, then come back to talk about it, which is exciting to hear.

*What is the compromise?*

The compromise is our house in Washington; we come back to the home base and our garden.

*Terry, I greatly appreciate your candidness in answering my questions.*

Well, it was fun, and The Macallan 18 single malt scotch helped, too!

*Whenever I see you at an ESA meeting, you always have a cloud of people hovering around you.*

Most of them are students; younger people. The students keep you young. Like I said, all my students want me for another 30 years. "You can't go!" [they say]. And I'm not!

## **Acknowledgment**

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# A large 28S rDNA-based phylogeny confirms the limitations of established morphological characters for classification of proteocephalidean tapeworms (Platyhelminthes, Cestoda)

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## Abstract

Proteocephalidean tapeworms form a diverse group of parasites currently known from 315 valid species. Most of the diversity of adult proteocephalideans can be found in freshwater fishes (predominantly catfishes), a large proportion infects reptiles, but only a few infect amphibians, and a single species has been found to parasitize possums. Although they have a cosmopolitan distribution, a large proportion of taxa are exclusively found in South America. We analyzed the largest proteocephalidean cestode molecular dataset to date comprising more than 100 species (30 new), including representatives from 54 genera (80%) and all subfamilies, thus significantly improving upon previous works to develop a molecular phylogeny for the group. The Old World origin of proteocephalideans is confirmed, with their more recent expansion in South America. The earliest diverging lineages are composed of Acanthotaeniinae and Gangesiinae but most of the presently recognized subfamilies (and genera) appear not to be monophyletic; a deep systematic reorganization of the order is thus needed and the present subfamilial system should be abandoned. The main characters on which the classical systematics of the group has been built, such as scolex morphology or relative position of genital organs in relation to the longitudinal musculature, are of limited value, as demonstrated by the very weak support for morphologically-defined subfamilies. How-

ever, new characters, such as the pattern of uterus development, relative ovary size, and egg structure have been identified, which may be useful in defining phylogenetically well-supported subgroups. A strongly supported lineage infecting various snakes from a wide geographical distribution was found. Although several improvements over previous works regarding phylogenetic resolution and taxon coverage were achieved in this study, the major polytomy in our tree, composed largely of siluriform parasites from the Neotropics, remained unresolved and possibly reflects a rapid radiation. The genus *Spasskyellina* Freze, 1965 is resurrected for three species of *Monticellia* bearing spinitriches on the margins of their suckers.

### Keywords

Eucestoda, Proteocephalidae, systematics, molecular phylogeny, host-parasite associations, *Spasskyellina*

### Introduction

Proteocephalideans (Platyhelminthes: Cestoda) form a morphologically homogeneous group of tapeworms found worldwide in freshwater fishes, reptiles, and amphibians (a single species is known from marsupial mammals). To our knowledge 315 valid species are currently known (unpublished), a large proportion of them being parasites of South American siluriform fishes (Freze 1965, Rego 1994).

Proteocephalideans historically formed their own order (Proteocephalidea with only one family, Proteocephalidae), the monophyly of which is strongly supported, but recent molecular analyses have placed them within a paraphyletic assemblage of ‘hooked’ tetraphyllidean cestodes (formerly Onchobothriidae), parasites of sharks and rays, which has led to the erection of a new order, the Onchoproteocephalidea by Caira et al. (2014). However, the lack of any morphological synapomorphies for this new order made this a somewhat controversial decision. For the purpose of the present paper, which is to study the internal relationships of the “terrestrial” onchoproteocephalideans (= proteocephalideans), this point is marginal and the new scheme proposed by Caira et al. (2014) is not considered further.

Previous attempts to study the interrelationships of proteocephalideans resulted in overall poorly resolved phylogenies. At the morphological level, the difficulty of defining reliable informative characters has prevented the construction of a stable taxonomic arrangement of the group (Rego 1994, 1995). The traditionally accepted families Proteocephalidae and Monticelliidae have been abandoned, and the whole group has been split into a number of subfamilies and genera, including the type genus *Proteocephalus* Weiland, 1858, which are sometimes obviously artificial because of their lack of synapomorphies and diversity of life-history traits (see de Chambrier et al. 2004c, 2009a). Molecular studies that have tried to resolve the proteocephalidean tree topology have largely been based on the variable domains (D1–D3) of the large nuclear ribosomal RNA subunit (28S rDNA), using increasingly larger datasets, i.e. 54 taxa analyzed by Zehnder and Mariaux (1999), and 75 taxa by de Chambrier et al. (2004c). Hypša et al. (2005) analyzed the phylogenetic relationships of only 52 taxa, but used sequences of three ribosomal RNA genes and the internal transcribed spacer 2 (ITS2). Additional molecular studies mostly considered questions at the specific/generic level [e.g. the *Proteocephalus* aggregate (Scholz

et al. 2007); African *Proteocephalus* (de Chambrier et al. 2011); *Testudotaenia* Freze, 1965 (de Chambrier et al. 2009a), Corallobothriinae (Rosas-Valdez et al. 2004, Scholz et al. 2011)] or employed only a very limited taxon sampling (e.g. Zehnder and de Chambrier 2000, Škeříková et al. 2001, de Chambrier et al. 2008, Scholz et al. 2013).

Although these studies have allowed for a better understanding of relationships within and between several subgroups, the major nodes of the proteocephalidean tree remain poorly supported, especially when considering the South American lineages. In the present contribution, an unprecedented collection of proteocephalidean samples have been gathered that includes the majority of all valid genera (54 out of 67), thus significantly increasing the taxon sampling within the order and adding representatives from previously unrepresented subfamilies. 28S rDNA sequences homologous to those published in studies by Zehnder and Mariaux (1999) and de Chambrier et al. (2004c) have been generated, and the newly generated data has been analyzed in conjunction with those previously published. Thus, the 28S rDNA data presented here represent the most comprehensive sampling of proteocephalideans to date.

## Methods

### Taxon sampling

The present study is based on the evaluation of a dataset of proteocephalideans collected during long-term studies carried out by the present authors and their co-workers, especially as part of research activities linked to the NSF-PBI project “A Survey of the Tapeworms (Cestoda: Platyhelminthes) from Vertebrate Bowels of the Earth” (2008–2014), which was aimed at mapping the global diversity of tapeworms. Despite significant sampling effort covering all zoogeographical regions and the most important host groups, the number of studied proteocephalideans that parasitize amphibians remains relatively small due to the paucity of cestodes in these hosts. In addition, several newly described proteocephalideans from the southern part of the Neotropical Region (Argentina) were not available for molecular studies. Among the 13 proteocephalidean genera that are not represented in our sampling, none presently contains more than two species (see Caira et al. 2012).

All taxa considered in this study are listed in Table 1. Most taxa included in de Chambrier et al. (2004c) are included in the present analysis; however, some taxonomical changes and novel identifications have taken place since this paper was published: *Proteocephalus pirarara* (Woodland, 1935a) is now *Scholzia emarginata* (Diesing, 1850); *Ophiotaenia* cf. *gallardi* is now *Ophiotaenia* sp.; *Pseudocrepidobothrium* sp. is now *Pseudocrepidobothrium ludovici* Ruedi & de Chambrier, 2012; *Megathylacus brooksi* Rego & Pavanelli, 1985 is now *Megathylacus jandia* (Woodland, 1934b); *Spatulifer* cf. *maringaensis* is now *Spatulifer maringaensis* Pavanelli & Rego, 1989. All but five molecular samples are vouchered, and in 86% of cases the vouchers are the holophore (sensu Astrin et al. 2013).

**Table 1.** Taxa used in the current study. Voucher numbers refer to the collections of the Natural History Museum of Geneva (MHNG-PLAT); Larry R Penner Parasitology Collection, Storrs, Connecticut, USA (LRP); Colección Nacional de Helminthos, México (CNHE); Collections of the Institute of Parasitology of the Czech Academy of Sciences (IPCAS). Out.: Outgroup. Type species are marked with a (\*) and hologenophores with an \*.

Species	Host species	Voucher number	Accession Number	Reference	Surface ovary %
<i>Acanthoactenia shipleyi</i> (T)	<i>Vanarus salvator</i>	*MHNG-PLAT-32887	AJ583453	de Chambrier et al. 2004c	6.8
<i>Ageneiosus brevifilis</i> (T)	<i>Ageneiosus inermis</i>	*MHNG-PLAT-21841	AJ388600	Zehnder et al. 1999	11.2
<i>Amphoteromorphus ninoi</i>	<i>Brachyplatystoma filamentosum</i>	*MHNG-PLAT-22239	AJ388624	de Chambrier et al. 2004c	11.7
<i>Amphoteromorphus periculus</i> (T)	<i>Brachyplatystoma rousseauxii</i>	*MHNG-PLAT-60052	KP729410	This paper	12.3
<i>Amphoteromorphus piraieba</i>	<i>Brachyplatystoma filamentosum</i>	MHNG-PLAT-22227	KP729407	This paper	12.5
<i>Amphoteromorphus piriformis</i>	<i>Brachyplatystoma rousseauxii</i>	*MHNG-PLAT-22211	AJ275231	de Chambrier et al. 2004c	12.5
<i>Australoactenia buntharagi</i>	<i>Enlydius enhydris</i>	*MHNG-PLAT-75447	KP729409	This paper	5.0
<i>Barsonella lafoni</i> (T)	<i>Clarias gariepinus</i>	*MHNG-PLAT-49399	FM955143	de Chambrier et al. 2009b	11.5
<i>Bryvela karuatayi</i> (T)	<i>Platymeriaichthys notatus</i>	*MHNG-PLAT-63128	KP729406	This paper	10.9
<i>Brooksiaella praeputialis</i> (T)	<i>Cetopsis coccutiens</i>	*MHNG-PLAT-21996	AJ275229	de Chambrier et al. 2004c	17.3
<i>Cangatiella arandasi</i> (T)	<i>Trachehyopterus galeatus</i>	*MHNG-PLAT-34736	KP729411	This paper	8.0
<i>Choanoscolex abcisus</i> (T)	<i>Pseudoplatystoma corruscans</i>	*MHNG-PLAT-17905	AJ388630	Zehnder et al. 1999	12.8
<i>Choanoscolex</i> sp.	<i>Pseudoplatystoma fasciatum</i>	*MHNG-PLAT-25102	AJ275064	de Chambrier et al. 2004c	5.1
<i>Corallobotrium solidum</i> (T)	<i>Malapterurus electricus</i>	*MHNG-PLAT-31553	AJ583450	de Chambrier et al. 2004c	7.2-7.4
<i>Corallobotrium</i> cf. <i>solidum</i>	<i>Malapterurus gosseii</i>	*MHNG-PLAT-63117	JN005780	Scholz et al. 2011	11.0
<i>Corallotenia intermedia</i>	<i>Ictalurus punctatus</i>	*MHNG-PLAT-25795	AJ275232	de Chambrier et al. 2004c	11.3
<i>Crepidobothrium geraldii</i> (T)	<i>Boa constrictor</i>	*MHNG-PLAT-66546	KC786018	Scholz et al. 2013	3.6
<i>Electroactenia malopteruri</i> (T)	<i>Malapterurus electricus</i>	*MHNG-PLAT-33995	JX477434	Ash et al. 2012	4.6-5.2
<i>Endorchis piraieba</i> (T)	<i>Brachyplatystoma filamentosum</i>	*MHNG-PLAT-21738	AJ388603	Zehnder et al. 1999	5.9
<i>Ephedrocephalus microcephalus</i> (T)	<i>Phractocephalus hemiolipterus</i>	*MHNG-PLAT-21910	AJ388605	Zehnder et al. 1999	11.4
<i>Essexiella fimbriata</i> (T)	<i>Ictalurus balsanus</i>	CNHE 4217	AY548162	Rosas Valdez et al. 2004	15.1
<i>Gangestia agracensis</i>	<i>Wallago attu</i>	*MHNG-PLAT-75457	JX477443	Ash et al. 2012	16.4
<i>Gangestia panasiluri</i>	<i>Silurus asotus</i>	*MHNG-PLAT-22436	AF286935	Olson et al. 2001	15.0
<i>Gibsoniella mandube</i> (T)	<i>Ageneiosus</i> sp.	*MHNG-PLAT-63119	KP729412	This paper	8.6
<i>Gibsoniella meursaulti</i>	<i>Ageneiosus inermis</i>	*MHNG-PLAT-21839	AJ388631	Zehnder et al. 1999	12.3
<i>Glantiaenia osculata</i> (T)	<i>Silurus glanis</i>	N/A	AJ388619	Zehnder et al. 1999	11.1

Species	Host species	Voucher number	Accession Number	Reference	Surface ovary %
<i>Gozeella siluri</i> (T)	<i>Prinampus pirinampu</i>	*MHNG-PLAT-21877	AJ388612	Zehnder et al. 1999	11.9
<i>Harriscolex kaparari</i> (T)	<i>Pseudoplatystoma tigrinum</i>	*MHNG-PLAT-22018	AJ275227	de Chambrier et al. 2004c	13.7
<i>Houssayella sudobim</i> (T)	<i>Sorubimichthys planiceps</i>	*MHNG-PLAT-62586	KP729404	This paper	9.7
<i>Jauella glandicephalus</i> (T)	<i>Zungaro jobu</i>	*MHNG-PLAT-31179	KP729399	This paper	9.6
<i>Kapsulotaenia</i> sp. 1	<i>Vananus rosenbergi</i>	*MHNG-PLAT-32842	AJ583452	de Chambrier et al. 2004c	5.5
<i>Kapsulotaenia</i> sp. 2	<i>Vananus gouldii</i>	*MHNG-PLAT-32839	AJ583455	de Chambrier et al. 2004c	3.5
<i>Kapsulotaenia</i> sp. 4	<i>Vananus varius</i>	*MHNG-PLAT-32838	AJ583454	de Chambrier et al. 2004c	6.5
<i>Macrobrithraenia ficata</i> (T)	<i>Xenopeltis unicolor</i>	*MHNG-PLAT-75454	KC786020	Scholz et al. 2013	4.1
<i>Manaosia bnacodemoca</i> (T)	<i>Sorubim lima</i>	*MHNG-PLAT-34186	KP729414	This paper	16.4
<i>Marsypocephalus heterobranchius</i>	<i>Heterobranchius bidorsalis</i>	*MHNG-PLAT-62973	KP729408	This paper	7.3
<i>Marsypocephalus rectangularis</i> (T)	<i>Clarias anguillaris</i>	*MHNG-PLAT-49366	KP729405	This paper	11.0
<i>Megathylacoides giganteum</i> (T)	<i>Ictalurus dugesi</i>	N/A	AY307117	Rosas Valdez et al. 2004	15.1
<i>Megathylacoides lamotheti</i>	<i>Ictalurus furcatus</i>	CNHE 4889	AY548165	Rosas Valdez et al. 2004	13.8
<i>Megathylacoides</i> sp.	<i>Ictalurus punctatus</i>	*MHNG-PLAT-35373	FM1956086	de Chambrier et al. 2009a	9.4
<i>Megathylacris jandia</i> (T)	<i>Zungaro zungaro</i>	*MHNG-PLAT-21874	AJ388596	Zehnder et al. 1999	8.6
<i>Monticellia coryphicephala</i> (T)	<i>Salminus brasiliensis</i>	*MHNG-PLAT-17984	AJ238832	Zehnder et al. 1999	18.5
<i>Nomimoscolex admonticellia</i>	<i>Prinampus pirinampu</i>	*MHNG-PLAT-21870	AJ388628	Zehnder et al. 1999	7.1
<i>Nomimoscolex chubbi</i>	<i>Gymnotus carapo</i>	*MHNG-PLAT-20351	AJ388625	Zehnder et al. 1999	7.7-12.4
<i>Nomimoscolex donad</i>	<i>Brachyplatystomaousseauxii</i>	*MHNG-PLAT-22269	AJ388613	Zehnder et al. 1999	7.5
<i>Nomimoscolex lenha</i>	<i>Sorubimichthys planiceps</i>	*MHNG-PLAT-21740	AJ388611	Zehnder et al. 1999	9.8
<i>Nomimoscolex lopesi</i>	<i>Pseudoplatystoma fasciatum</i>	*MHNG-PLAT-21963	AJ388618	Zehnder et al. 1999	8.8
<i>Nomimoscolex matogrossensis</i>	<i>Hoplias malabaricus</i>	*MHNG-PLAT-17913	AJ388614	Zehnder et al. 1999	12.2-14.5
<i>Nomimoscolex piraebea</i> (T)	<i>Brachyplatystoma capapretum</i>	*MHNG-PLAT-22284	AJ388608	Zehnder et al. 1999	10.6-12.8
<i>Nomimoscolex sudobim</i>	<i>Pseudoplatystoma fasciatum</i>	*MHNG-PLAT-21969	AJ388597	Zehnder et al. 1999	12.0
<i>Nomimoscolex suspectus</i>	<i>Brachyplatystoma vaillantii</i>	*MHNG-PLAT-22298	AJ388602	de Chambrier et al. 2004c	6.2-10.2
<i>Nupelia portoricensis</i> (T)	<i>Sorubim lima</i>	*MHNG-PLAT-34185	KP729401	This paper	10.3
<i>Ophiotaenia bungari</i>	<i>Bungarus fasciatus</i>	*MHNG-PLAT-75442	KC786022	Scholz et al. 2013	3.1
<i>Ophiotaenia europaea</i>	<i>Natrix maura</i>	*MHNG-PLAT-18407	AJ388598	Zehnder et al. 1999	12.7
<i>Ophiotaenia filaroides</i>	<i>Ambystoma tigrinum</i>	*MHNG-PLAT-63372	KP729416	This paper	11.5

Species	Host species	Voucher number	Accession Number	Reference	Surface ovary %
<i>Ophiotaenia gallardi</i>	<i>Pseudechis porphyriacus</i>	*MHNG-PLAT-36550	KC786025	Scholz et al. 2013	3.2
<i>Ophiotaenia grandis</i>	<i>Agkistrodon piscivorus</i>	N/A	AJ388632	Zehnder et al. 1999	2.1
<i>Ophiotaenia javana</i>	<i>Bothrops jararaca</i>	*MHNG-PLAT-12393	AJ388607	Zehnder et al. 1999	2.4
<i>Ophiotaenia lapata</i>	<i>Madagascarophis colubrina</i>	*MHNG-PLAT-79567	KC786021	Scholz et al. 2013	2.8
<i>Ophiotaenia pambiodex</i>	<i>Causus maculatus</i>	*MHNG-PLAT-25962	AJ388620	Zehnder et al. 1999	4.2
<i>Ophiotaenia panguayensis</i>	<i>Hydrodynastes gigas</i>	*MHNG-PLAT-16927	AJ388629	Zehnder et al. 1999	3.3
<i>Ophiotaenia</i> cf. <i>perspicua</i>	<i>Nerodia rhombifer</i>	*MHNG-PLAT-35370	KP729415	This paper	2.3
<i>Ophiotaenia sanbernardinensis</i>	<i>Felicops leopardinus</i>	*MHNG-PLAT-18251	AJ388637	Zehnder et al. 1999	5.0
<i>Ophiotaenia saphena</i>	<i>Lithobates pipiens</i>	*MHNG-PLAT-32851	KP729402	This paper	8.3-8.7
<i>Pangasiocestus romani</i> (T)	<i>Pangasius larnaudii</i>	*MHNG-PLAT-75449	KP729397	This paper	10.6
<i>Paraproteocephalus panasilari</i> (T)	<i>Silurus asotus</i>	*MHNG-PLAT-22438	AJ388604	Zehnder et al. 1999	4.3
<i>Peltidocotyle lenha</i>	<i>Zungaro zungaro</i>	*MHNG-PLAT-22373	AJ238837	Zehnder et al. 1999	14.7
<i>Peltidocotyle rugosa</i> (T)	<i>Pseudoplatystoma reticulatum</i>	*MHNG-PLAT-22374	AJ238835	Zehnder et al. 1999	13.9-14.7
<i>Postgangesia inarmata</i>	<i>Silurus glanis</i>	*MHNG-PLAT-34212	AM931032	de Chambrier et al. 2008	12.5
<i>Proteocephalidae</i> gen. sp.	<i>Amia calva</i>	*MHNG-PLAT-35548	FM1956088	de Chambrier et al. 2009a	9.3
<i>Proteocephalus ficicollis</i>	<i>Gasterosteus aculeatus</i>	*MHNG-PLAT-24081	AJ388636	Zehnder et al. 1999	16.3
<i>Proteocephalus fluviatilis</i>	<i>Micropterus dolomieu</i>	IPCAS C-364	KP729390	This paper	17.0
<i>Proteocephalus glanduligerus</i>	<i>Clarias</i> sp.	*MHNG-PLAT-50013	KP729392	This paper	9.8
<i>Proteocephalus gobiorum</i>	<i>Neogobius fluviatilis</i>	IPCAS C-299	KP729393	This paper	19.7
<i>Proteocephalus hemiolipteri</i>	<i>Phractocephalus hemiolipterus</i>	*MHNG-PLAT-21889	AJ388622	Zehnder et al. 1999	11.8
<i>Proteocephalus keyukiayu</i>	<i>Preodon granulosus</i>	*MHNG-PLAT-66572	KP729388	This paper	Immature
<i>Proteocephalus longicollis</i>	<i>Coregonus lavaretus</i>	*MHNG-PLAT-21681	AJ388626	de Chambrier et al. 2004c	13.3
<i>Proteocephalus macrocephalus</i>	<i>Anguilla anguilla</i>	N/A	AJ388609	Zehnder et al. 1999	18.3
<i>Proteocephalus macroballus</i>	<i>Cichla monoculus</i>	MHNG-PLAT-36526	KP729394	This paper	6.0-6.6
<i>Proteocephalus midoriensis</i>	<i>Lefua echigonia</i>	MHNG-PLAT-22431	AJ388610	Zehnder et al. 1999	19.4
<i>Proteocephalus percae</i>	<i>Perca fluviatilis</i>	*MHNG-PLAT-36744	AJ388594	Zehnder et al. 1999	13.8
<i>Proteocephalus perplexus</i>	<i>Amia calva</i>	*MHNG-PLAT-35366	FM1956089	de Chambrier et al. 2009a	12.0
<i>Proteocephalus pinguis</i>	<i>Esox lucius</i>	*IPCAS C-679	KP729395	This paper	9.6
<i>Proteocephalus pleoglossi</i>	<i>Plecoglossus altivelis</i>	MHNG-PLAT-22434	AJ388606	de Chambrier et al. 2004c	7.4
<i>Proteocephalus renaudi</i>	<i>Platydonis costatus</i>	*MHNG-PLAT-17894	AJ388638	Zehnder et al. 1999	7.1

Species	Host species	Voucher number	Accession Number	Reference	Surface ovary %
<i>Proctocephalus sagittus</i>	<i>Barbatula barbatula</i>	IPCAS C-33	KP729391	This paper	13.4
<i>Proctocephalus sulcatus</i>	<i>Clavotus laticeps</i>	MHNG-PLAT-54150	KP729396	This paper	10.6
<i>Proctocephalus synodontis</i>	<i>Synodontis caudivittatus</i>	*MHNG-PLAT-62931	JN005778	Scholz et al. 2011b	9.2-13.0
<i>Proctocephalus tetrastomus</i>	<i>Hypomesus nipponensis</i>	MHNG-PLAT-22429	AJ388635	Zehnder et al. 1999	7.0-11.4
<i>Proctocephalus</i> sp.	<i>Ictalurus punctatus</i>	*MHNG-PLAT-36278	FM956085	de Chambrier et al. 2009a	11.0
<i>Pseudocrepidobothrium eivasi</i> (T)	<i>Phractocephalus hemioliopterus</i>	MHNG-PLAT-27431	AJ388623	de Chambrier et al. 2004c	11.6
<i>Pseudocrepidobothrium ludovici</i>	<i>Phractocephalus hemioliopterus</i>	*MHNG-PLAT-22108	AJ275063	Zehnder et al. 1999	9.7-10.3
<i>Regoella brevis</i> (T)	<i>Pseudoplatus reticulatum</i>	*MHNG-PLAT-79184	KP729389	This paper	11.5
<i>Ritaceus ritaii</i> (T)	<i>Rita rita</i>	*MHNG-PLAT-63242	JX477447	Ash et al. 2012	17.7
<i>Rostellotenia nilotica</i> (T)	<i>Varanus niloticus</i>	*MHNG-PLAT-34195	KP729398	This paper	7.0
<i>Rostellotenia</i> sp.	<i>Varanus exanthematicus</i>	MHNG-PLAT-25026	AJ388593	de Chambrier et al. 2004c	3.9
<i>Rudolphiella piracatinga</i>	<i>Calophrys macropterus</i>	*MHNG-PLAT-19868	AJ388627	Zehnder et al. 1999	10.4
<i>Rudolphiella szidati</i>	<i>Lucioperleodus pati</i>	*MHNG-PLAT-24668	AJ388617	de Chambrier et al. 2004c	14.4
<i>Sandonella sandoni</i> (T)	<i>Heterotis niloticus</i>	*MHNG-PLAT-49356	AM931033	Unpublished	8.8
<i>Scholia emarginata</i> (T)	<i>Phractocephalus hemioliopterus</i>	*MHNG-PLAT-22106	KC786016	Scholz et al. 2013	10.8-15.9
<i>Sciadocephalus megalodiscus</i> (T)	<i>Cichla monoculus</i>	MHNG-PLAT-37332	KP729403	This paper	N/A
<i>Silurotenia siluri</i> (T)	<i>Silurus glanis</i>	MHNG-PLAT-25027	AJ388592	Zehnder et al. 1999	14.8
<i>Spaskevella lenha</i> (T)	<i>Sorubimichthys planiceps</i>	*MHNG-PLAT-69600	KP729413	This paper	9.8
<i>Spaskevella spinulifera</i>	<i>Pseudoplatus corruscans</i>	*MHNG-PLAT-34216	KP729417	This paper	10.1
<i>Spatulifer maringaensis</i>	<i>Sorubim lima</i>	*MHNG-PLAT-21986	AJ388634	de Chambrier et al. 2004c	17.4
<i>Testudotenia testudo</i> (T)	<i>Apalone spinifera</i>	*MHNG-PLAT-35320	FM956082	de Chambrier et al. 2009a	6.2
<i>Thaumasocolex didelphidis</i> (T)	<i>Didelphis marsupialis</i>	*MHNG-PLAT-28993	AJ275065	de Chambrier et al. 2004c	8.4
<i>Travassouella jandia</i> (T)	<i>Zungaro jabru</i>	MHNG-PLAT-31175	KP729400	This paper	8.6-10.7
<i>Vernaia pseudotropii</i> (T)	<i>Clupisoma garua</i>	*MHNG-PLAT-63247	JX477453	Ash et al. 2012	3.3
<i>Zygobothrium megalophthalmi</i> (T)	<i>Phractocephalus hemioliopterus</i>	*MHNG-PLAT-21846	AJ388621	Zehnder et al. 1999	20.8
[Out.] <i>Acanibothrium</i> sp.	<i>Dayatis longus</i>	LRP-2112	AF286953	Olson et al. 2001	N/A
[Out.] <i>Phyllbothrium lactuca</i>	<i>Mustelus asterias</i>	LRP-2115	AF286960	Olson et al. 2001	N/A
[Out.] <i>Tetraphyllidea</i> gen. sp.	<i>Squalus acanthias</i>	N/A	AJ388591	Zehnder et al. 1999	N/A

## Molecular phylogenetic analyses

Total genomic DNA extraction, PCR amplification, and sequencing were done as outlined in Scholz et al. (2013). Eighty-three published and 30 newly generated 28S rDNA sequences were combined and analysed in conjunction (see Table 1 for GenBank accession numbers and further details). *Acanthobothrium* sp. ('Onchoproteocephalidea'), *Phyllobothrium lactuca* Beneden, 1850 (Phyllobothriidea) and "Tetraphyllidea" gen. sp. were used as outgroup taxa. Sequences were aligned with MAFFT (Multiple Alignment using Fast Fourier Transform, <http://www.ebi.ac.uk/Tools/msa/mafft/>) using the default settings. An alignment mask excluding sites of uncertain positional homology was generated using ZORRO (Wu et al. 2012). ZORRO uses a pair Hidden Markov Model and a weighted sum of pairs scheme (guided by a reference tree) that sums up the probability that a given alignment column appears over the total alignment landscape, thus providing an objective estimate of whether positions consist of correctly aligned, homologous residues. Default settings were used except for the invocation of the – sample option; positions with confidence scores < 0.4 were excluded from subsequent analyses. MRMODELTEST v. 2.3 (Nylander 2004) was used to select models of sequence evolution using the Akaike Information Criterion. Bayesian inference (BI) analysis was performed using MRBAYES version 3.2 (Ronquist and Huelsenbeck 2003) using the GTR model of sequence evolution with proportion of invariant sites and gamma-distributed rate variation amongst sites (nst = 6, rates = invgamma). Default prior settings and heating schemes were used. Two parallel runs were performed for 10,000,000 generations and sampled every 1,000 generations. The burn-in was defined as the point at which the average standard deviation of split frequencies were < 0.01. Consensus trees were constructed using the 50% majority rule and nodes with < 0.95 posterior probabilities (pp) were collapsed. Leaf-stability tests, implemented in P4 (Foster 2004), were carried out to identify unstable taxa. Given a set of trees, for each set of four taxa, the frequency of the four possible resolutions of quartets was calculated. For each taxon, the highest percentages for quartets including that taxon were averaged and listed as "Maximum". Therefore, unstable taxa across the trees were considered to be those that have lower average maximum percentages. In this study, the three taxa with the lowest "Maximum" values were eliminated from analyses in order to increase nodal support for the remaining groupings (Wilkinson 1996).

## Morphological analysis

Taxonomic identification was performed on specimens fixed and mounted on microscope slides according to de Chambrier (2001). Uterine development was characterized according to de Chambrier et al. (2004c) but a new "intermediate type" was recognized and is described below (see Fig. 2). The relative size of the ovary, i.e. the ovary to proglottid surface ratio, was calculated for each species according to the

method described in de Chambrier et al. (2012). Approximate values might be due to inaccurate drawings or fixation methods reported by the original authors. Eggs were examined in distilled water.

## Data Resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at <http://dx.doi.org/10.5061/dryad.dv44b>.

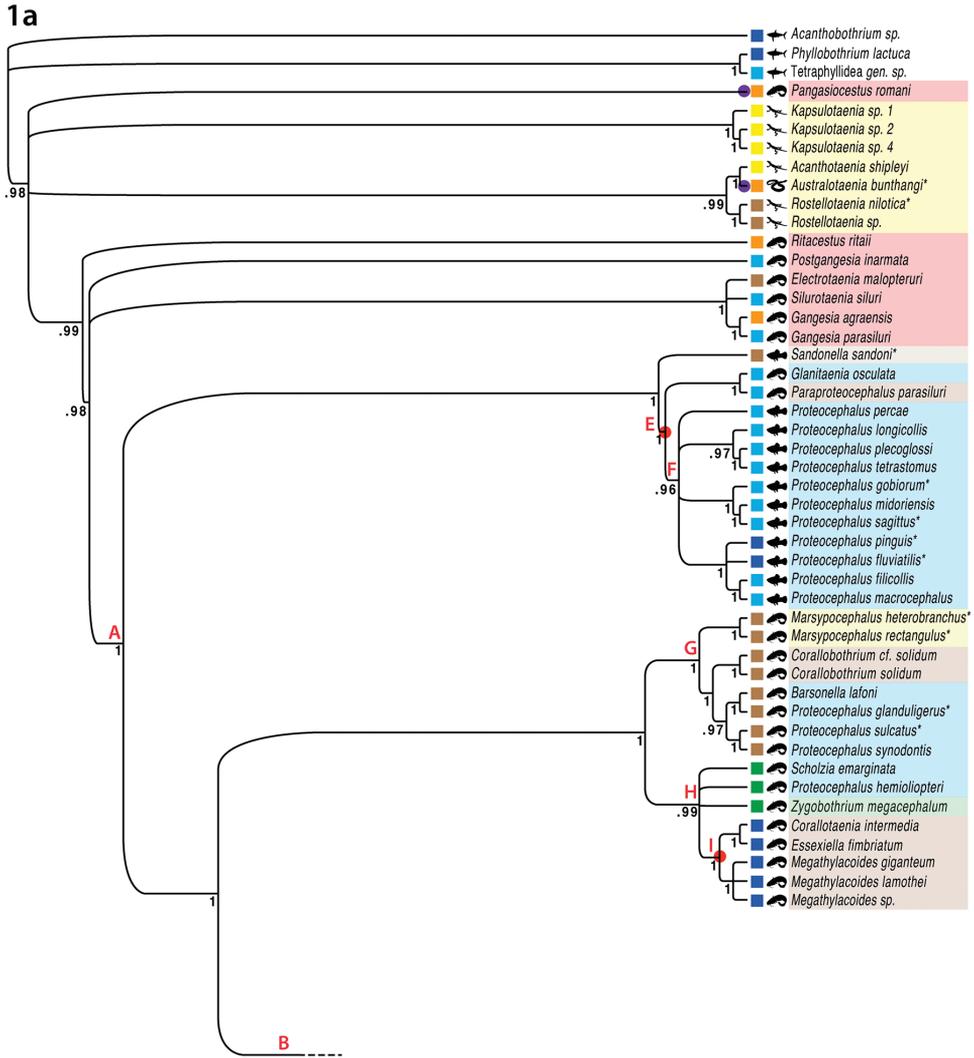
## Results

### Molecular phylogeny

The complete 28S rDNA dataset comprised 110 ingroup taxa (from 54 genera, representing all 13 currently recognized subfamilies) and three outgroup taxa. Importantly, 46 genera were represented by their type species (see Table 1). The alignment consisted of 1937 characters of which 420 were excluded, leaving 1517 for the analyses.

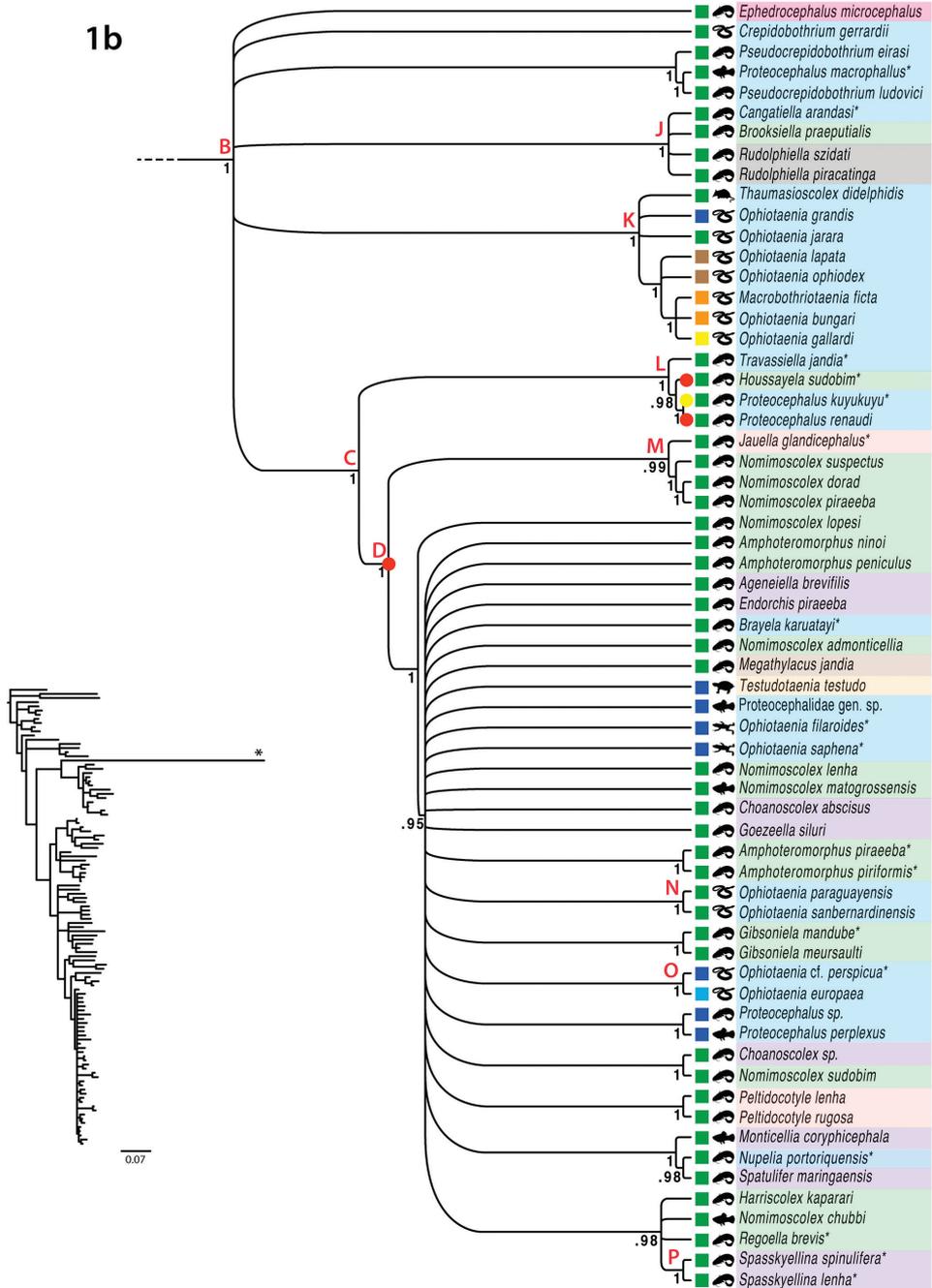
In an initial BI analysis, several nodes had posterior probabilities (pp) < 0.95, resulting in a tree with only 60 well-supported nodes (see Suppl. material 1: Fig.1). In order to identify unstable taxa for subsequent exclusion, a leaf stability test was conducted. This revealed *Vermaia pseudotropii* (Verma, 1928), *Sciadocephalus megalodiscus* Diesing, 1850 and *Manaosia bracodemoca* Woodland, 1935 to be the least stable taxa (see Suppl. material 2: Table 1). Curiously, the position of the longest branching taxon, *Sandonella sandoni* (Lynsdale, 1960), was very stable (Fig. 1b inset; Suppl. material 1: Fig.1, Suppl. material 2: Table 1). The positions of the excluded taxa were as follows: *Vermaia pseudotropii* was in an unresolved position at the base of the tree, *Sciadocephalus megalodiscus* was in an unresolved position in a clade composed of the ingroup taxa to the exclusion of Gangesiinae Mola, 1929 and Acanthotaeniinae Freze, 1963, and *Manaosia bracodemoca* was in an unresolved position in the large subclade of *Clade D* (Suppl. material 1: Fig.1).

In a subsequent BI analysis, in which the above-mentioned three taxa had been excluded, three nodes had improved support ( $\geq 0.95$  pp), resulting in 63 well-supported nodes in total (Fig. 1a, b). Thus, further topology descriptions are based on the better-supported tree in which nodes of particular interests were labeled *Clades A–P* (Fig. 1a, b). Specifically, those better-supported nodes concern the positions of (i) *Postgangesia inarmata* de Chambrier, Al-Kallak & Mariaux, 2003, (ii) *Ritacestus ritaii* (Verma, 1926), and (iii) the sister-group relationship between *Choanoscolex* sp. and *Nomimoscolex sudobim* Woodland, 1935 (Fig. 1a, b; Suppl. material 1: Fig.1). Thus, the Gangesiinae were shown to be non-monophyletic except for a clade composed of *Electrotaenia malopteruri* (Fritsch, 1886), *Silurotaenia siluri* (Batsch, 1786) and *Gangesia* spp. (Fig. 1a).



Acanthotaeniinae	Peltidocotylinae	elasmobranchs	Afrotrropic
Corallobothriinae	Proteocephalinae	catfishes	Australasia
Ephedrocephalinae	Rudolphiellinae	non siluriform fishes	Indomalaya
Gangesiinae	Sandonellinae	amphibians	Palearctic
Marsypocephalinae	Testudotaeniinae	lizards	Nearctic
Monticelliinae	Zygobothriinae	snakes	Neotropic
Nupeliinae		turtles	
		possums	

**Figure 1.** Bayesian inference of partial (domains 1–3) 28S rDNA sequences of a reduced taxon set of proteocephalideans (unstable taxa *Sciadocephalus megalodiscus*, *Vermaia pseudotropii* and *Manaosia bracedemoca* have been removed) performed using MrBayes version 3.2 using the GTR + I + G model of sequence evolution. Two parallel runs were performed for 10,000,000 generations; 4,000,000 generations were discarded as burnin. Branches with posterior probability (pp) support below 95% are collapsed; pp



are indicated below branches. Asterisks mark new sequences. Red letters A to P refer to specific nodes discussed in the text. Red circles refer to the acquisition of “Type 2” uterus development; purple circles: acquisition of “intermediate type” uterus development; yellow circle: uterus development unknown (see Discussion). A mute phylogram of the same tree is inserted and the long branch leading to *Sandonella sandoni* is marked with an asterisk.

The three earliest diverging lineages were formed of *Pangasiocestus romani* Scholz & de Chambrier, 2012 and the Acanthotaeniinae, where the Acanthotaeniinae were possibly non-monophyletic, split into a monophyletic *Kapsulotaenia* Freze, 1965, and a monophyletic assemblage of *Acanthotaenia shipleyi* + *Australotaenia bunthangi* + *Rostellotaenia* spp. (posterior probability = 0.88; not shown), but where all three lineages took an unresolved position at the base of the tree.

The Gangesiinae formed three paraphyletic lineages composed of *Ritacestus ritaii*, *Postgangesia inarmata*, and a clade composed of *Electrotaenia malopteruri*, *Silurotaenia siluri* and *Gangesia* spp. (Fig. 1a), to the exclusion of the remainder of the tree (*Clade A*).

The remainder of the tree (*Clade A*) was structured as follows: The earliest diverging group consisted of *Sandonella sandoni* (Lynsdale, 1960) which parasitizes an ancient osteoglossiform fish in Africa and which formed the sister group to *Clade E*. The latter was composed of two monotypic sister taxa *Glanitaenia* de Chambrier, Zehnder, Vaucher & Mariaux, 2004 (Proteocephalinae) and *Paraproteocephalus* Chen in Dubinina, 1962 (Corallobothriinae), both of which parasitize silurid catfishes in the Palearctic Region. These, in turn, formed the sister group to *Clade F*, which was composed of the *Proteocephalus* aggregate (see de Chambrier et al. 2004c) from Holarctic teleosts, including two newly added species from North America, *P. fluviatilis* Bangham, 1925 and *P. pinguis* La Rue, 1911.

The next well-supported group structured of *Clade G*, which was exclusively composed of taxa from African siluriforms belonging to three subfamilies (Corallobothriinae, Marsypocephalinae and Proteocephalinae), and which formed the sister group to *Clade H*. The latter was composed of *Scholzia emarginata*, *Proteocephalus hemioliopteri* de Chambrier & Vaucher, 1997 and *Zygobothrium megacephalum* Diesing, 1850, all of which are anatomically similar parasites of the Neotropical catfish *Phractocephalus hemioliopterus* (Bloch & Schneider, 1801), but which are traditionally placed in different subfamilies, and of a monophyletic group of Nearctic proteocephalideans (*Clade I*), all parasitizing channel catfish (Ictaluridae); members of *Clade I* are placed in the Corallobothriinae because they possess a metascolex.

The most derived assemblage, *Clade B*, remained largely unresolved, with five early diverging lineages composed of (i) *Ephedrocephalus microcephalus* Diesing, 1850, (ii) *Crepidobothrium gerrardii* Monticelli, 1900, (iii) a clade of *Pseudocrepidobothrium* spp. + *Proteocephalus macrophallus* (Diesing, 1850), (iv) *Clade J*, composed of *Rudolphiella* spp. + *Cangatiella arandasi* Pavanelli & Machado dos Santos, 1991 + *Brooksiella praeputialis* (Rego, Santos & Silva, 1974), and (v) *Clade K*, composed of *Ophiotaenia* spp., *Macrobothriotaenia ficta* (Meggitt, 1931), all parasites of snakes from various zoogeographical regions, and *Thaumasioscolex didelphidis* Cañeda-Guzmán, de Chambrier & Scholz, 2001, the only proteocephalidean found in possums; (i)–(iv) were exclusively from the Neotropics.

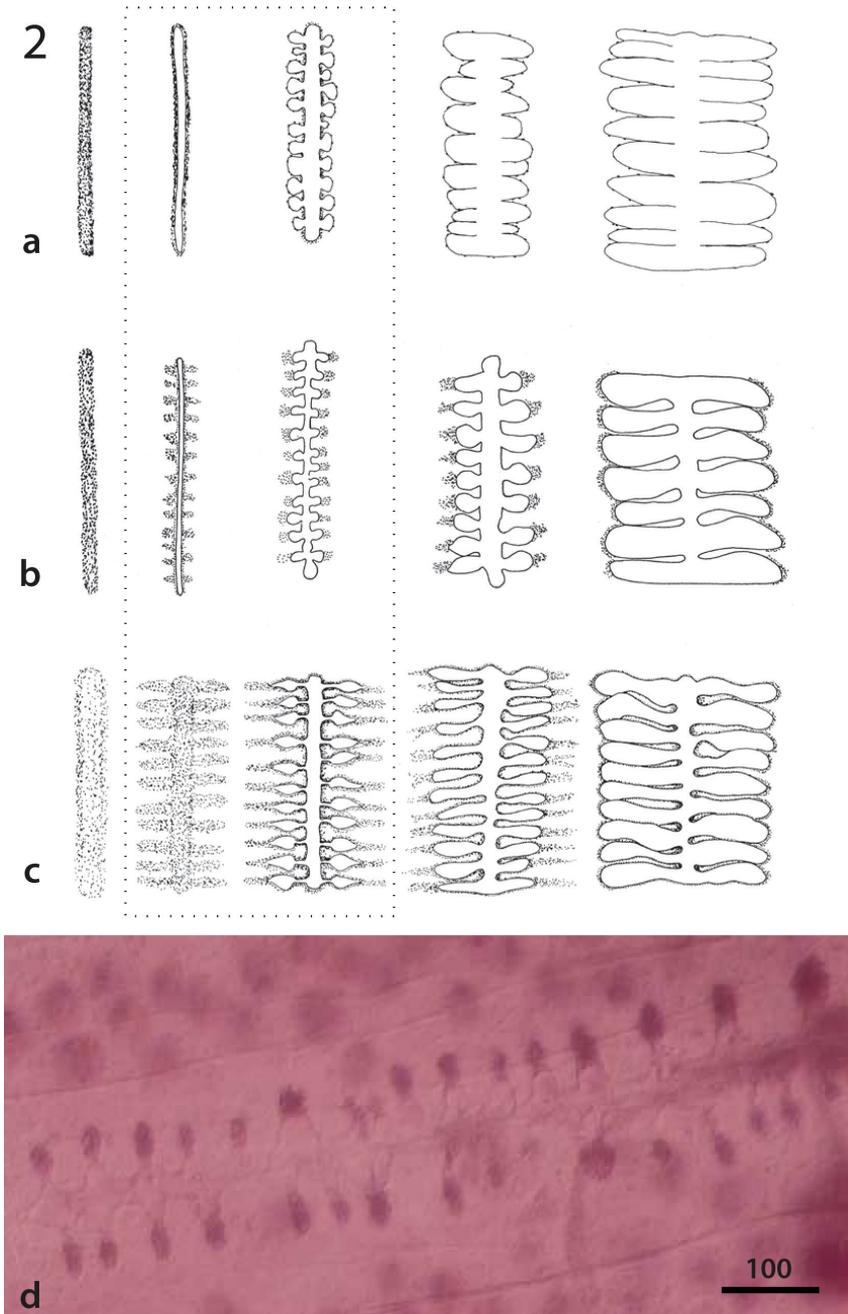
The large polytomy found in *Clade C* was, to a large degree, composed of proteocephalideans parasitizing South American fishes (predominantly siluriforms of the families Pimelodidae, Auchenopteridae and Doradidae). *Clade L* formed the earliest diverging lineage of *Clade C* and was composed of *Travassielia jandia* (Woodland, 1934), *Houssayela sudobim* (Woodland, 1935) and *Proteocephalus kuyukuyu* Wood-

land, 1935 and *P. renaudi* de Chambrier & Vaucher, 1994. The sister group to the large polytomy in *Clade C* was formed of *Clade M*, which included *Jauella glandicephalus* Rego & Pavanelli, 1985, *Nomimoscolex suspectus* Zehnder, de Chambrier, Vaucher & Mariaux, 2000, *N. dorad* (Woodland, 1935) and *N. piraebea* Woodland, 1934. The remainder of *Clade C* formed largely a comb which comprised, amongst others, *Testudotaenia testudo* (Magath, 1924), a parasite of North American soft-shelled turtles and bowfin (*Amia calva*), a clade of *Proteocephalus* sp. and *Proteocephalus perlexus* La Rue, 1911, parasitizing North American catfish and bowfins respectively, two distinct clades of *Ophiotaenia* La Rue, 1911, *Clade N* (parasites of South American snakes) and *Clade O* (parasites of European and Nearctic snakes), and two unresolved *Ophiotaenia* species, *O. filaroides* La Rue, 1909 and *O. saphena* Osler, 1931, parasitizing North American salamanders and frogs, respectively.

The possible monophyly of 17 proteocephalidean genera could be examined, at least preliminarily, because two or more species of these genera were included in our analyses (numerous proteocephalidean genera are monotypic or species-poor). According to the current taxon sampling, the following genera, listed alphabetically, appeared monophyletic (the numbers in parentheses indicate the total number of species sequenced and the number of distinct lineages in which species of a given genus appeared): *Corallobothrium* Fritsch, 1886 (2/1), *Gangesia* Woodland, 1924 (2/1), *Gibsoniella* Rego, 1984 (2/1), *Kapsulotaenia* Freze, 1965 (3/1), *Marsypocephalus* Wedl, 1861 (2/1), *Megathylacoides* Jones, Kerley & Sneed, 1956 (3/1), *Peltidocotyle* Diesing, 1850 (2/1), *Proteocephalus* aggregate (11/1), *Rostellotaenia* Freze, 1963 (2/1) and *Spasskyellina* Freze, 1965 (2/1) (see discussion below for the latter). The monophyly of *Rudolphiella* Fuhrmann, 1916 (2/1) was not rejected by these results. In contrast, *Pseudocrepidobothrium* Rego & Ivanov, 2001 (2/2) is paraphyletic and the genera *Amphoteromorphus* Diesing, 1850 (4/3), *Choanoscolex* La Rue, 1911 (2/2), *Nomimoscolex* Woodland, 1934 (9/7), *Ophiotaenia* (12/10) and *Proteocephalus* (20/7) appeared to be polyphyletic based on their current classification.

## Morphological analysis

At the morphological level, the ovary to proglottid surface ratio ranged between 2.0% in *Ophiotaenia grandis* La Rue, 1911 to 20.8% in *Zygobothrium megacephalum* (Table 1). Examination of new whole mounts also identified a novel form of the uterine development in addition to those described by de Chambrier et al. (2004c). This development is characterized as follows: in immature proglottids, the uterine stem forms an elongated concentration of chromophilic cells; in premature proglottids the chromophilic cells concentrate in areas where lateral uterine extensions will develop; in mature proglottids, a tubular uterine stem appears and develops small thin-walled lateral diverticula topped with a conspicuous concentration of numerous intensely stained cells; in pregravid and gravid proglottids, the lateral diverticula grow and eventually occupy most of the proglottid width (Fig. 2b, d). We call this development “intermediate type”.



**Figure 2.** Schematic representation of proteocephalidean uterus development (**a–c**). The uterus observed in early immature, premature, mature, pregravid and gravid proglottids is represented from left to right. The major differences are observed in premature and mature proglottids (dotted line): **a** and **c** Development of Type 1 and 2, respectively (de Chambrier et al. 2004c) **b** Development of an “intermediate type” as observed in *Pangasiocestus* and *Australotaenia* (this paper) **d** Typical “intermediate type” uterus in a mature proglottid of *Australotaenia bunthangi* de Chambrier & Scholz, 2012 (holotype, MHNG-PLAT-75447). Scale in micrometers.

## Discussion

Since the publications of de Chambrier et al. (2004c) and Hypša et al. (2005), no attempt has been made to unravel the phylogenetic structure of proteocephalideans. Two immediate observations can be made when comparing our results to the de Chambrier et al. (2004c) tree: (1) an overall better resolution is achieved with the increased taxon sampling; and (2) all clades that were supported in de Chambrier et al. (2004c) remain so in these results. However, a number of differences can also be noted as discussed below.

### Early diverging lineages – *Acanthotaeniinae* and *Gangesiinae*

In both de Chambrier et al. (2004c) and the present study, the *Gangesiinae* from Siluriformes, mostly in Indomalaya and Palearctic (but with one species in Afrotropics), and *Acanthotaeniinae* from reptiles in Australasia, Afrotropic and Indomalaya are early diverging lineages. However, their order is now reversed with the *Acanthotaeniinae*, together with *Pangasiocestus romani* (*Gangesiinae*), taking the earliest diverging position. Thus, the present results suggest either the paraphyly of the subfamily or the necessity to handle *Pangasiocestus* Scholz & de Chambrier, 2012 as an independent lineage. This monotypic genus was initially placed in the *Gangesiinae* based on its scolex morphology, which is characterized by a large rostellum-like apical organ. However, it differs from all *gangesiinae* in a number of morphological characteristics. These include the peculiar, rosette-like scolex with a large, discoidal apical organ devoid of hooks; a very weakly-developed inner longitudinal musculature, which does not form bundles (unlike those of other *gangesiinae* genera, which form numerous bundles of muscle fibers; see Scholz et al. 1999, de Chambrier et al. 2003, de Chambrier et al. 2004b, Ash et al. 2012 for more details); and the variable size of testes, which are considerably smaller and denser in the lateral than in the median field. These morphological features support the separation of *Pangasiocestus* from the *Gangesiinae*, as shown by our genetic analysis, despite the superficial resemblance of its scolex with that of other *gangesiinae* cestodes.

It should also be noted that, together with *Australotaenia* de Chambrier & de Chambrier, 2010, *Pangasiocestus* has a particular, intermediate development of the uterus (see below), that contrasts that of all other *Gangesiinae* and *Acanthotaeniinae*, which have a Type 1 development of the uterus. *P. romani* was found in a catfish in Cambodia, and species of *Australotaenia* are distributed in Australia and Indomalaya, which would suggest an Old World origin for proteocephalideans. This scenario is consistent with the results of de Chambrier et al. (2004c) and contradicts the hypothesis of Brooks (1978), who favored a South American origin of the group.

### The *Proteocephalus* aggregate and the enigmatic *Sandonella* and *Sciadocephalus*

The position of *Sandonella* Khalil, 1960 as a separate long-branching lineage, as already observed by de Chambrier et al. (2008), was confirmed in the present study. *Sandonella*

formed the sister group to the strongly supported *Clade E*, which is composed of species of the *Proteocephalus* aggregate (*Clade F*) that are parasites of teleosts in the Holarctic Region, and monotypic genera *Glanitaenia* and *Paraproteocephalus*, which are parasites of silurid catfish in the Palearctic Region. The members of the *Proteocephalus* aggregate (= *Proteocephalus* sensu stricto) will retain the generic name since this clade undoubtedly includes *P. ambiguus* (Dujardin, 1845), the type species of *Proteocephalus*, as shown by Scholz et al. (2007). The addition of two *Proteocephalus* species of Nearctic origin [*P. fluviatilis* from centrarchids (Perciformes) and *P. pinguis* from pikes (Esociformes)] to the dataset revealed their affinity with the *Proteocephalus* aggregate. This close phylogenetic relationship of the Palearctic and Nearctic taxa analyzed is in accordance with their similar morphology (Freze 1965, Scholz and Hanzelová 1998). The diversity of hosts in *Clade E* is surprising when compared to other subgroups of proteocephalideans that generally diversify in discrete groups of catfish. In this case a Holartic radiation of these cestodes in multiple groups of fishes has occurred.

*Sandonella sandoni* was placed in a new genus and subfamily, Sandonellinae, mostly because of the characteristic posterior position of its vitellarium, which is unique among proteocephalideans and somewhat resembles that of the Cyclophyllidea in being formed by two compact, yet deeply lobulated postovarian masses near the posterior margin of the proglottids (Khalil 1960, see also fig. 6 in de Chambrier et al. 2008). Bâ and Marchand (1994) observed the unique structure of *S. sandoni* spermatozoa (with a single axoneme) and de Chambrier et al. (2008) reported its widespread presence in *Heterotis niloticus* (Cuvier, 1829) throughout Africa and described additional original morphological characters such as a scolex with a highly modified apical structure formed by 4 muscular retractile lappets, a dilated, vesicle-like proximal part of the external sperm duct, a unique morphology of the uterus, and a complex proglottization with mixed smaller and larger (wider) proglottids. Despite these peculiarities, as well as its derived 28S sequence, the position of *S. sandoni* as a sister group of Holarctic Proteocephalinae was established by de Chambrier et al. (2008) and is not questioned by these results. The presence of this relatively derived parasite in a basal fish lineage (Osteoglossiformes) is further evidence that the evolution of proteocephalideans does not closely match that of their hosts. It should be noted though that the phylogenetic position of this taxon has not yet been tested in more global cestode phylogenies (i.e. Waeschenbach et al. 2012, Caira et al. 2014).

*Sciadocephalus megalodiscus* parasitizing *Cichla monoculus* Agassiz, 1831 (Perciformes) in the Neotropical region and described by Diesing (1850) is another enigmatic taxon. In its redescription Rego et al. (1999) noted several peculiar morphological features, such as an umbrella-shaped metascolex, a uterus rapidly resolving into capsules, and a musculature with numerous isolated longitudinal fibers, and placed the species in the Corallobothriinae based on the presence of a metascolex [which is, however, a homoplastic character (Scholz et al. 2013)] and the medullary position of the genital organs. In our initial evaluation, this taxon appeared as the earliest diverging lineage of *Clade A* (see Suppl. material 1: Fig.1) but it has also been identified as one of the three least stable taxa in the analysis and had therefore been excluded from fur-

ther analyses. Nevertheless, this possible distinct position of the species among proteocephalideans, supported by its combination of peculiar morphological characteristics, might justify its future placement in a separate, higher taxonomic group.

### **African fish proteocephalideans**

Our considerably enlarged dataset of fish proteocephalideans from Africa covers most of their diversity and includes all genera reported from the Afrotropical Region. It revealed that all but one species (the gangesiine *Electrotaenia malopteruri* – see above) from African siluriform fish form a well-supported, relatively basal *Clade G*. This is one of the most important novelties of the present study: species placed in three subfamilies are phylogenetically closely related despite important morphological differences. These are: i) the Corallobothriinae (two species of *Corallobothrium* including its type species from malapterurid electric catfish) characterized mainly by a well-developed metascolex and medullary testes; ii) the Marsypocephalinae (two species from clariids) with a simple scolex and cortical testes; and iii) the Proteocephalinae (three *Proteocephalus* species from clariid, claroteid and mochokid catfish, and *Barsonella lafoni* de Chambrier, Scholz, Beletew & Mariaux, 2009 from *Clarias* spp.), with a relatively simple scolex and medullary testes (de Chambrier et al. 2009b). This grouping of taxa with markedly different scoleces as well as conspicuously distinct position of the testes (medullary versus cortical) is further evidence that morphological characteristics related to the scolex and internal topology of genital organs are homoplastic and should be interpreted with great caution. A similar situation was demonstrated in *Macrobothriotaenia ficta*, a snake parasite from Indomalaya, which possesses a tetraphyllidean-like scolex: it is closely related to species of *Ophiotaenia* with a simple scolex (Scholz et al. 2013; see also *Clade K*), but less so with *Thaumasioscolex didephidis* despite having a very similar scolex morphology. The new results also indicate that zoogeography and host associations may have played a much more important role in the evolutionary history of proteocephalidean cestodes than previously thought (Freze 1965, Rego et al. 1998).

### **Parasites of the Neotropical pimelodid catfish *Phractocephalus hemioliopterus***

Neotropical catfish, in particular pimelodids, harbour the highest number of species (and genera) of proteocephalidean cestodes. However, these parasites do not form a monophyletic assemblage, even though most of them belong to our most derived clade with unresolved internal relationships (see also Zehnder and Mariaux 1999, de Chambrier et al. 2004c). The current study confirmed the polyphyly of these cestodes, including the markedly distant position of three species from the pimelodid catfish *Phractocephalus hemioliopterus* (*Clade H*) from the remaining cestodes parasitizing other siluriforms from South America, as first observed in a much smaller dataset by Hypša et al. (2005).

As many as six species reported from *P. hemioliopterus* were included in our analyses. Three of them, namely *Proteocephalus hemioliopteri*, *Scholzia emarginata* (both Proteocephalinae) and *Zygobothrium megacephalum* (Zygobothriinae), differ markedly from each other in their scolex morphology (see de Chambrier et al. 2005), yet form a well-supported lineage (*Clade H*) together with Nearctic “corallobothriines” (*Clade I*). Their phylogenetic position is, thus, more basal and distant from that of other proteocephalideans parasitizing Neotropical teleosts.

The remaining three taxa that parasitize *P. hemioliopterus*, i.e. two species of *Pseudocrepidobothrium* (Proteocephalinae) and *Ephedrocephalus microcephalus* Diesing, 1850 (Ephedrocephalinae) group in an unresolved position towards the base of the South American radiation. This suggests possible independent colonizations of this host. The basal position of these parasites is in accordance with the fact that *P. hemioliopterus* is one of the most ancient pimelodids, as suggested by fossil records dating from Middle to Late Miocene (Lundberg and Littmann 2003).

Our data do not enable any reliable assessment regarding a possible host-parasite coevolution, especially in the case of pimelodid catfishes and their Neotropical proteocephalideans. A comparison of the interrelationships of the Pimelodidae based on robust morphological and molecular evidence (Lundberg et al. 2011 and references therein) with the present data does not reveal any obvious pattern of possible co-evolutionary history. In fact, cestodes from closely related pimelodids such as species of *Pseudoplatystoma* Bleeker, 1862 and *Sorubimichthys planiceps* (Spix & Agassiz, 1829) are unrelated and belong to distant lineages (Table 1 and Fig. 1a, b).

### Nearctic “corallobothriines” from channel catfishes (Ictaluridae)

Nearctic species from channel catfish form a well-supported, monophyletic lineage (*Clade I*) composed of species of three genera, *Essexiella* Scholz, de Chambrier, Mariaux & Kuchta, 2011, *Megathylacoides* and *Corallotaenia* Freze, 1965. However, the Nearctic genera, conventionally placed in the Corallobothriinae because they possess a metascolex, are not closely related to the monotypic *Corallobothrium* from the electric catfish, *Malapterurus electricus* Gmelin, 1789, in Africa and their morphological resemblance is probably a result of convergent evolution (Scholz et al. 2011). In fact, the subfamily Corallobothriinae groups species of unrelated genera (African *Corallobothrium* in *Clade G*, three Nearctic genera in *Clade I*, Japanese *Paraproteocephalus* in *Clade E* and Neotropical *Megathylacus* Woodland, 1934 in *Clade D* – Fig. 1a, b) that share apparently homoplasious morphological characteristics, i.e. a well-developed metascolex and a medullary position of genital organs as described above (Freze 1965, Rego 1994, Rosas-Valdez et al. 2004).

As a consequence, a new taxon should be proposed to accommodate Nearctic channel catfish proteocephalideans, which are apparently unrelated either to the true corallobothriines (in fact now represented by *C. solidum* and a species to be described, both from Africa) or to the various other proteocephalideans from freshwater teleosts

in North America that are distributed throughout the phylogenetic tree (*Clades F and D* – see Fig. 1a, b). Similarly, the position of *Paraproteocephalus* within the Corallobothriinae will need to be reconsidered. This placement is likely to be due to convergences in scolex shape, and the genus should be placed in the Proteocephaliinae.

### **Cosmopolitan reptilian proteocephalideans**

The distribution of proteocephalideans in snakes is particularly interesting. Multiple colonizations of reptiles, as already suggested by de Chambrier et al. (2004c), are confirmed here and at least three main events (see *Clades K, N and O*) are shown in this study (besides the case of *Australotaenia*). In each case, cestodes of snakes appear to be related to various proteocephalideans of Neotropical catfishes and other teleosts (Fig. 1a, b). The most interesting novel insight from our study in this context is the strong support found for *Clade K*, composed almost exclusively of parasites from snakes (Viperidae, Elapidae, Lamprophiidae and Xenopeltidae) throughout the world (with the exception of Palearctic) and the unique switch to a mammalian host (*Didelphidius marsupialis* L., 1758) in the northernmost Neotropical Region in the case of *Thaumasioscolex didelphidis*. Colubridae are notably absent from this host list. This grouping of rather derived snake parasites cannot be unambiguously explained by our data and may either be the sign of a relatively recent colonization of unrelated groups in all continents or a trace of a very ancient colonization of snakes. Even though all these species belong to the Proteocephalinae because of the medullary position of their genital organs and the absence of a metascolex, they actually differ markedly from each other, especially in their scolex morphology, and were placed in three separate genera (Freze 1965, de Chambrier 1989a, de Chambrier 1989b, Rego 1994, Cañeda-Guzmán et al. 2001, Scholz et al. 2013). Two of these (*Macrobothriotaenia* Freze, 1965 and *Thaumasioscolex*) are essentially characterized by peculiar scoleces. The position of *Crepidobothrium gerrardii* (Monticelli, 1900), a parasite of Boidae that is also characterized by a distinctive scolex, is not fully resolved but is possibly unrelated to this radiation.

Species of *Ophiotaenia* in colubrids from Holarctic (2 species – *Clade O*), Neotropical dipsadids (2 species – *Clade N*), and Nearctic amphibians are possibly unrelated and appear within a polytomy composed of numerous lineages of Neotropical fish proteocephalideans. They are morphologically uniform and do not differ significantly from the other species of *Ophiotaenia* in *Clade K*, as all of them have a similar scolex and strobilar morphology, including relative ovary size (see de Chambrier et al. 2012 and Table 1). However, members of the larger radiation (*Clade K*) have a Type 1 uterus whereas those in the other clades have a Type 2 uterus. Consequently, and as suspected (Ammann and de Chambrier 2008), it is clear that *Ophiotaenia* is a composite genus and this name should be restricted to species of *Clade O*, which includes the type species *Ophiotaenia perspicua* La Rue, 1911 from Nearctic colubrids. Species in *Clade O* have proportionally larger ovaries than those in the remaining species of “*Ophiotaenia*” (*Clades K, N*), which will need to be allocated to other (new) genera.

## “Neotropical fish” superclade

In addition to the above-mentioned “reptilian” lineages, our derived *Clade B* is composed of a number of Neotropical parasites of catfishes and a few other teleosts, where the highest species richness can be found in the Pimelodidae (Siluriformes) (de Chambrier and Vaucher 1999, Rego et al. 1999). A few parasites from amphibians and turtles, as well as *Proteocephalus perplexus* La Rue, 1911 from bowfin (*Amia calva* L., 1766), also belong to this large polytomy. de Chambrier et al. (2009a) showed that *Testudotaenia* Freze, 1965 of the monotypic subfamily Testudotaeniinae was part of a North American clade of proteocephalid parasites of fishes despite its distinctive morphology. These results do not contradict this hypothesis although *Testudotaenia*'s closest relatives cannot be inferred from the present tree.

Despite our enlarged sample size, the present study did not resolve the relationships of most Neotropical proteocephalideans from teleosts, and in this respect does not significantly improve the results of Zehnder and Mariaux (1999), de Chambrier et al. (2004c) or Hypša et al. (2005). Still, some nodes are now well supported, e.g., species of *Brooksiella* Rego, Chubb & Pavanelli, 1999, *Rudolphiella* and *Cangatiella* Pavanelli & Machado dos Santos, 1991 (*Clade J*), species of *Travassiwella* Rego & Pavanelli, 1987, *Houssayella* Rego, 1987 and two species of “*Proteocephalus*” (*Clade L*), and three species of the largely polyphyletic *Nomimoscolex*, including *N. piraeeba* (type species), together with *Jauella glandicephalus* (*Clade M*). However, these well-supported lineages are composed of species with dissimilar morphologies and often belong to different subfamilies (as many as three in *Clade J*). In addition, they parasitize fish of different genera, families or even orders, which makes it impossible to define them logically for now.

Other molecular markers, possibly large mtDNA fragments, as used by Waeschenbach et al. (2012), are obviously needed if the internal phylogenetic structure of the derived *Clade B* is to be unravelled, although the possibility that this node represents a hard-polytomy should also be considered. A similar situation, i.e. support for some of the internal nodes but a lack of support for the major lineages, was observed for the Caryophyllidea, another order of fish tapeworms, despite the use of several nuclear and mitochondrial markers. These commonly employed molecular markers did not contain sufficient phylogenetic signal due to substitution saturation (Brabec et al. 2012).

Catfishes (order Siluriformes) represent one of the key host groups for proteocephalidean cestodes, but there is no obvious coevolutionary pattern between them. This lack of closer host-associations at a higher taxonomic level is not surprising because catfishes form an extraordinarily diverse group of teleosts with over 3,000 valid recognized species (Eschmeyer et al. 2004). The interrelationships of large groups in the Siluroidei, which comprises almost all catfish hosts of proteocephalideans, including the Neotropical pimelodids and heptapterids (Pimelodoidea) and African taxa (“Big Africa” clade with cestode-hosting families Mochokidae, Malapteruridae, and Auchenoglanidae and phylogenetically distant Clariidae) are poorly resolved (Sullivan et al. 2006). Molecular data suggest an ancient siluriform presence, if not origin, in South America, but phylogenies inferred from *rag* gene sequences did not identify any African-South American catfish clade (Sullivan et al. 2006).

## Monophyly/polyphyly of proteocephalidean genera

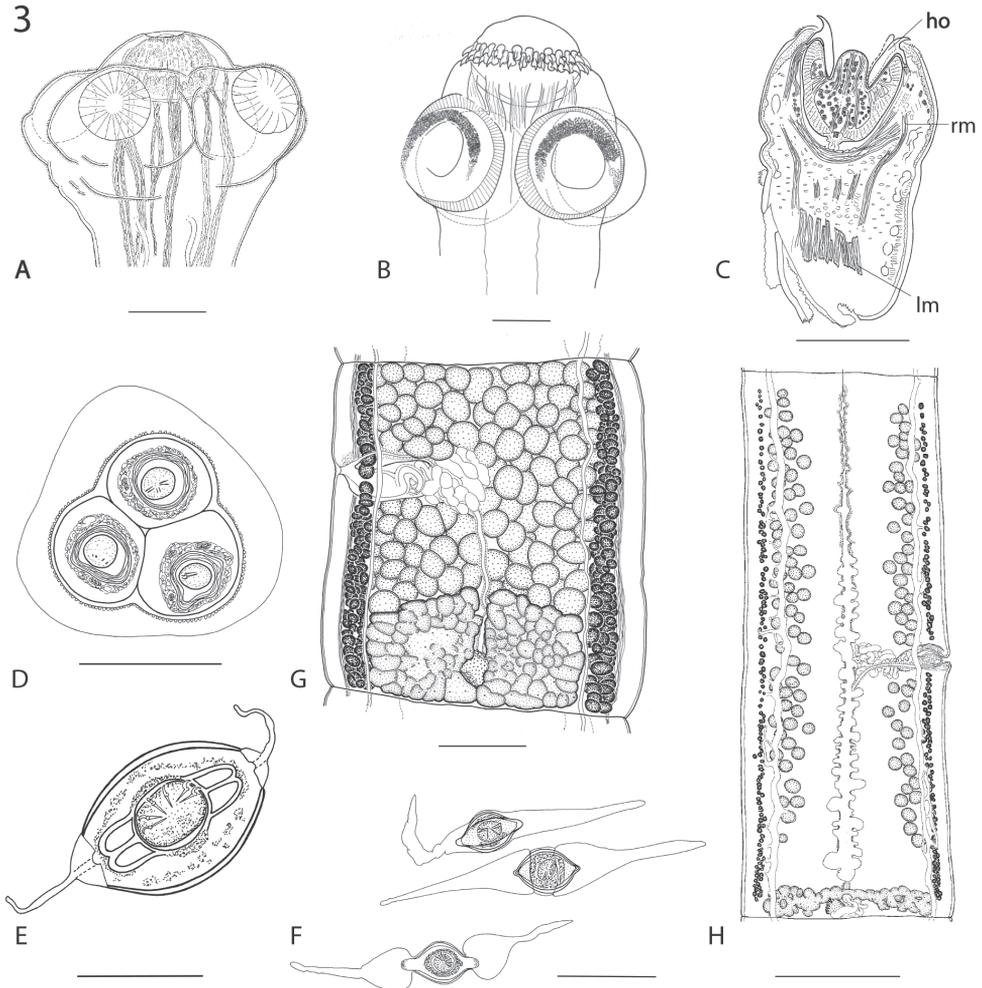
Even though 10 genera (see above) appeared to form monophyletic assemblages, all but one (*Proteocephalus* aggregate) were represented by a very low number of species (2–3), and the validity of some of them may still have to be reconsidered when a denser sampling is available. In contrast, all species-rich genera with at least nine species analyzed (*Nomimoscolex*, *Ophiotaenia* and *Proteocephalus* sensu lato), as well as *Amphoteromorphus* (4 species), appeared to be polyphyletic and are distributed across numerous lineages, even though their morphology and host-associations are quite similar.

A situation comparable to that of *Proteocephalus* (species of this genus belong to at least 7 distinct lineages – Fig. 1a, b) starts to emerge in *Nomimoscolex*. As previously noted by Zehnder et al. (2000), our *Nomimoscolex* samples are distributed across several distinct lineages in *Clade D*. The type species *N. piraeeba*, belonging to the well-supported *Clade M*, and all *Nomimoscolex* loosely grouped across other lineages in *Clade D* will ultimately have to be placed in other genera. At this point, however, objective morphological characters are still lacking to recognize these worms.

This work also confirms the polyphyly of *Monticellia* La Rue, 1911 in its present form with *M. spinulifera* Woodland, 1935 and *M. lenha* Woodland, 1933 found in siluriforms forming well-supported *Clade P*, which is distantly related to the type species of the genus, *M. coryphicephala* (Monticelli, 1891) from characids. The two former species belong to *Monticellia* since de Chambrier and Vaucher (1999) synonymised *Spasskyellina* Freze, 1965 with *Monticellia*. *Spasskyellina* was later considered as valid by de Chambrier et al. (2006), without considering the 1999 work, thus generating confusion about the status of the genus. Given the obvious morphological support that confirms our molecular results, we propose splitting *Monticellia* in order to reflect this situation and to formally resurrect here the genus *Spasskyellina*, that was erected in 1965 by Freze (Freze 1965) for those taxa possessing gladiate spinitriches (de Chambrier and Scholz 2008, Chervy 2009) on margins of their suckers, i.e. *Spasskyellina lenha* (Woodland, 1933) Freze, 1965 (type species) and *Spasskyellina spinulifera* (Woodland, 1935a) Freze, 1965. They are presented under this name in Fig. 1b. Additionally, *Spasskyellina mandi* Pavanelli & Takemoto, 1996 is confirmed in this revalidated genus because of its obviously similar morphology, contrary to previous observations (Pavanelli and Takemoto 1996, de Chambrier and Vaucher 1999). Since molecular data for other species of *Monticellia* are not available, they are provisionally kept in that genus.

## Evolution of morphological characters

Regarding the evolution of morphological characters, the most obvious and evolutionarily important observation derived from Fig. 1a, b is the presence of a rostellar apparatus with retractor muscles in all the basal taxa. Such structures (Fig. 3A–C), although with some variation, are characteristic of all Acanthotaeniinae and Gangesiinae and are lost in all more derived Proteocephalidae (*Clade A*) without exception. Although apical



**Figure 3. A–C** Scoleces with rostellum-like organs and retractor muscles. **A** Without hooks. *Ritacestus ritaii* (Verma, 1926) (modified from de Chambrier et al. 2011) **B** With hooks. *Gangesia bengalensis* (Southwell, 1913) (modified from Ash et al. 2012) **C** Partly-invaginated. Sagittal section, ho: hooks; rm: retractor muscles; lm: longitudinal muscles. *Vermaia pseudotropii* (Verma, 1928) (modified from Ash et al. 2010) **D–F** Egg modifications **D** Egg cluster in a capsule. *Vandiermeria beveridgei* (de Chambrier & de Chambrier, 2010) (modified from de Chambrier and de Chambrier 2010) **E** Egg with two polar projections. *Brooksiella praeputialis* (Rego, Santos & Silva, 1974) (modified from de Chambrier et al. 2004a) **F** Eggs with two polar projections. *Rudolphiella* spp. from *Calophrys macropterus* (two eggs above) and *Megalonema platanum*, respectively (modified from Gil de Pertierra and de Chambrier 2000) **G–H** Ovary size **G** Relatively large ovary (16.4% proglottid surface) in *Gangesia agraeensis* Verma, 1928 (modified from Ash et al. 2012) **H** Relatively small ovary in *Ophiotaenia lapata* Rabeloson, Ranaivoson & de Chambrier (2012) (2.8% of proglottid surface) (modified from Rabeloson et al. 2012). Scale-bars: **A, B, C** = 100  $\mu$ m; **D, E** = 20  $\mu$ m; **F** = 50  $\mu$ m; **G** = 200  $\mu$ m; **H** = 500  $\mu$ m.

structures are present in some other members of the order such as in the *Proteocephalus* aggregate from the Holarctic (see Scholz et al. 1998), *P. sophiae* de Chambrier & Rego, 1994 from South America, *P. glanduligerus* (Janicki, 1928) from Africa, *Jauella* Rego & Pavanelli, 1985 or *Nomimoscolex* sensu stricto as defined by Zehnder et al. (2000) (*Clade M*), these are very different, especially because they lack a supporting muscular apparatus (retractors) (de Chambrier and Rego 1994, de Chambrier and Vaucher 1999, Scholz et al. 2009). This kind of functional simplification, in this case due to the loss of apical attachment structures, is known from other cestode groups and has appeared repeatedly, for example in a number of derived cyclophyllidean genera (Jones et al. 1994), even though these structures are unlikely to be homologous.

The development of the uterus seems to represent one of the key features that reflects the evolution of proteocephalideans and characterizes their major lineages. The evolution of uterine structure as described in de Chambrier et al. (2004c) is essentially supported in the present analysis although with some added complexity. Both putative acquisitions of Type 2 uterine development observed by these authors are observed in our extended analysis (see red circles in *Clade E* and *D*) but the inclusion of new taxa revealed a third instance of transition of this character in *Clade I* in a well-supported group of Nearctic Corallobothriinae. Furthermore, the situation for taxa belonging to *Clade L* is unclear with two of them harbouring a Type 2 uterus, one a Type 1 uterus (*TravassIELla jandia*) and one with missing information ( gravid proglottids of *Proteocephalus kuyukuyu* have never been found).

Two basal taxa belonging to Acanthotaeniinae and Gangesiinae show a different, as yet undescribed, form of uterus development that we call “intermediate type” (see purple circles on Fig. 1a). This development differs from Type 1 development by the presence of chromophilic cells at points of origin of the lateral extensions of the uterus before the lateral stems are visible. It differs from Type 2 development in an early appearance of the main tubular uterus axis (Fig. 2). Assuming that the “intermediate type” might be a transitional stage between both uterus development types, a possible interpretation of this observation would be that a general trend toward the acquisition of Type 2 uterus development exists throughout the proteocephalidean diversity.

New morphological characters that are potentially useful for proteocephalidean taxonomy are notoriously difficult to define. However, Ammann and de Chambrier (2008) observed differences in the relative surface area of the ovary in relation to the total surface of the proglottids (see Fig. 3G–H). In their study, this ratio was on average five times lower in 27 species of *Ophiotaenia* from snakes in the New World compared to Palearctic members of the *Proteocephalus* aggregate from teleosts. More recently, de Chambrier et al. (2012) compared 66 of the nominal species of *Ophiotaenia* from Old and New World reptilian hosts with 69 species of *Proteocephalus* from freshwater teleosts. They noted that the ovaries of species parasitic in non-Palearctic snakes are proportionally smaller than those in species of *Proteocephalus* parasitic in teleost fishes from all over the world and also considerably smaller than that of congeneric species from European hosts.

In the present study, data on the relative size of the ovary are provided for all taxa analyzed (see Table 1). Results from two former studies (Ammann and de Chambrier 2008, de Chambrier et al. 2012) are verified here in the context of a larger dataset covering more genera and subfamilies. We can conclude that the ratio of the ovary surface to the proglottid surface in mature proglottids largely corresponds to major host groups and thus represents a promising character of possible phylogenetic importance that should be routinely reported in future descriptions or redescriptions of proteocephalidean taxa (for methodology of taking this ratio – see de Chambrier et al. 2012). However, patterns in the relative size of the ovary of species from different host groups discussed above are not universal and notable exceptions exist. For example, the smallest known ovary is found in *Margaritaella gracilis* Arredondo & Gil de Pertierra, 2012 from the catfish *Callichthys callichthys* (L., 1758) (ratio of 0.6–1.8%; Arredondo and Gil de Pertierra 2012) and not in a species from snakes.

Characters related to eggs and their morphology have been shown to be important in the systematics of proteocephalidean cestodes (Gil de Pertierra and de Chambrier 2000, Scholz and de Chambrier 2003, de Chambrier et al. 2005, de Chambrier 2006, de Chambrier and de Chambrier 2010, Scholz et al. 2011) but have generally been underexploited and remain poorly known for many species. Here, they allow the characterization of a well-supported node grouping species of *Rudolphiella*, *Brooksiella* and *Cangatiella* (Clade J), because all these taxa possess very typical eggs with polar extensions (Fig. 3E, F). To our knowledge, no other proteocephalidean shows such egg characteristics and thus the presence of polar extensions can be considered as a synapomorphy that defines this group. Furthermore, species in these genera all present a ventral vitellarium and *Brooksiella* and all species of *Rudolphiella* (but not *Cangatiella*) have a folliculate ovary and a metascolex (Gil de Pertierra and Viozzi 1999, de Chambrier et al. 2004b). These morphological characteristics seem to strongly support this clade.

Another kind of egg (in capsules) (Fig. 3D) is found in the basal Australasian *Kapsulotaenia* parasites of varanids and is also known in *Vandiermenia* de Chambrier & de Chambrier, 2010 and some “*Ophiotaenia*” of Australian snakes. In the Neotropics a similar evolution of eggs (in groups of 4–6) is known in *Thaumasioscolex*, the single known proteocephalidean of marsupials. The phylogenetic value of this character remains presently doubtful as some of these worms belong to isolated clades (Scholz et al. 2013). It may however represent an interesting convergent adaptation in proteocephalidean with terrestrial life cycle, although it curiously did not seem to have appeared outside of the Australasian (and maybe Neotropical) region despite the presence of terrestrial proteocephalideans in other areas.

Unfortunately, most lineages revealed in the present study lack such obvious synapomorphies due to a high degree of homoplasy across numerous morphological characters previously used for distinguishing individual genera and subfamilies, such as scolex morphology and the position of reproductive organs in relation to the inner longitudinal musculature (Rego 1994, 1999). Thus, the delineation of many taxonomic groups using morphological features remains currently impossible.

## Conclusions

This study is based on the most representative molecular dataset of proteocephalidean taxa ever sampled (33% of all valid species, almost 80% of genera and all extant subfamilies). However, some groups are still under-represented, mainly because of the difficulties in obtaining fresh samples, either due to their low prevalence and the protection or rare occurrence of their hosts. Probably the most serious gap in our dataset is the small number (two species) of proteocephalideans parasitizing amphibians (frogs and salamanders). These are usually extremely rare, with less than 1% of host infected (de Chambrier et al. 2006, Marsella and de Chambrier 2008). Similarly, none of the four species of *Ophiotaenia* from lizards (excluding *Varanus* spp.) were available for this analysis. In contrast, our geographical coverage was rather comprehensive thanks to the intensive sampling effort during the last decades. This considerably enlarged dataset has helped to better characterize several lineages, but the relationships of many taxa, especially those in the most derived *Clade B*, largely comprising parasites of catfishes in the Neotropical Region, remain largely unresolved.

The evolutionary history of the order has been apparently much more complicated than one would expect, considering a relatively small number (about 315) of extant species. Although we did not formally examine the host-parasite coevolution of proteocephalideans here, our tree strongly suggests the occurrence of several colonization events of poikilothermic vertebrates as well as repeated colonization of the principal zoogeographical regions with the most recent, and probably explosive, radiation in Neotropical teleosts, especially pimelodid catfishes.

Based on 28S rDNA sequences, these results support several new insights into the evolution of proteocephalideans. Unfortunately, they also cast a number of doubts on our present understanding of the classifications within this group: most recognized subfamily-level taxa are, at best, only partially supported. A notable consequence is that scolex morphology and the position of internal organs (testes, uterus and vitelline follicles in relation to the inner longitudinal musculature) should be considered with caution when used for higher-level taxonomy, i.e. to distinguish genera and subfamilies. Clearly a complete taxonomical reorganization of the order is needed. This will likely include the designation of a number of well-supported families and the removal of the subfamilial terminology. Any formal reorganization of the order, however, would be premature as long as a more complete multigene analysis remains to be performed. At lower taxonomical levels, we nevertheless propose resurrecting the genus *Spasskyellina* for three species of *Monticellia* (see above) but, for now, we consider that further nomenclatural adaptations should be delayed until clearly supported groups, reinforced by well-defined morphological characters, can be named and adequately characterized.

Results reported herein make it obvious that a new classification should not be based on the characters traditionally used for circumscribing genera and families (Rego 1994). Instead, new synapomorphies should be found to distinguish morphologically similar, but genetically distinct lineages, and to propose a more natural classification that would better reflect the evolutionary history of proteocephalideans. If applied, this

would represent a clear change of strategy in our attempts to understand the evolution of the group. In practice, this could lead to the erection of numerous small genera consisting of a few species each and sharing only a few morphological, possibly discrete, synapomorphies but with good molecular support. A careful move in that direction might be the future of the systematics and taxonomy of proteocephalideans.

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## **Supplementary material 1**

### **Figure 1**

Authors: Alain de Chambrier, Andrea Waeschenbach, Makda Fisseha, Tomáš Scholz, Jean Mariaux

Data type: Phylogenetic tree

Explanation note: Bayesian inference of partial (domains 1–3) 28S rDNA sequences of the complete taxon set of proteocephalideans performed using MrBayes version 3.1 using the GTR + I + G model of sequence evolution. Two parallel runs were performed for 10,000,000 generations; 8,000,000 generations were discarded as burnin. Branches with posterior probability (pp) support below 95% are collapsed; pp are indicated below branches.

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## **Supplementary material 2**

### **Table 1**

Authors: Alain de Chambrier, Andrea Waeschenbach, Makda Fisseha, Tomáš Scholz, Jean Mariaux

Data type: Leaf stability test results

Explanation note: Leaf stability test results from the post-burnin posterior tree distribution from two MrBayes runs that included the full complement of taxa. Taxa are ranked based on their positional stability estimated from the Maximum, which is an average of all the highest percentages from all possible quartet sets for a particular taxon, Difference, which is the difference between the highest and the second highest percentages from all possible quartet sets for a particular taxon, and Entropy, which is calculated as the normalized sum of logs for each quartet percentages (except the unresolved polygamy).

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# A new gall crab species (Brachyura, Cryptochiridae) associated with the free-living coral *Trachyphyllia geoffroyi* (Scleractinia, Merulinidae)

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## Abstract

A new species of gall crab is described from the free-living stony coral *Trachyphyllia geoffroyi*. Specimens were collected during field work in Lembeh Strait (Indonesia) and off Kudat (Malaysian Borneo). This new species, here named *Lithoscaptus semperi* sp. n., is the ninth species assigned to the genus. It can be separated from its congeners by not having the internal orbital angle extending beyond the external orbital angle, and by the stout female P2 merus with prominent distomesial projection. In addition, the carapace surface appears smooth, despite having small tubercles on the anterior half, and is without noticeable spines, other than those on the frontal margin. The distinctive carapace pattern in life is a diagnostic character in male specimens.

## Keywords

Cospeciation, host specificity, Indonesia, Malaysia, Thoracotremata

## Introduction

During field work in Indonesia and Malaysia an undescribed gall crab species was encountered living in dwellings in free-living *Trachyphyllia geoffroyi* (Audouin, 1826) corals. This scleractinian species is usually found on soft substrate of reef bases near coral reefs, where it can occur in large numbers (Fisk 1983, Best and Hoeksema 1987). The polyps of *T. geoffroyi* are fleshy and a large mantle can extend beyond the perimeter of the skeleton.

*Trachyphyllia geoffroyi* was classified in its own family, Trachyphylliidae Verrill, 1901, but this taxon was recently synonymised with Merulinidae Verrill, 1865 (Huang et al. 2014). The sister genera of *Trachyphyllia* Milne Edwards & Haime, 1849 are *Coelestrea* Verrill, 1866 and *Dipsastraea* de Blainville, 1830, which include coral species that formerly belonged to *Goniastrea* Milne Edwards & Haime, 1848 and *Favia* Milne Edwards, 1857. Corals belonging to these genera are host to cryptochirids of the genus *Lithoscaptus* A. Milne-Edwards, 1862 (Fize and Serène 1957, Kropp 1990).

Semper (1881) mentioned gall crabs associated with Indo-Pacific and Atlantic “*Trachyphyllia*”, but no formally described gall crab has been recorded living in association with *T. geoffroyi*. This new gall crab species, here named *Lithoscaptus semperi* sp. n., is the ninth assigned to the genus.

## Methods

Gall crabs were collected in Indonesia (Lembah Strait, N Sulawesi – February 2012) and Malaysia (off Kudat, N Borneo – September 2012). Corals were searched for gall crabs, taken to the field laboratory and subsequently split with hammer and chisel. The crabs were preserved in 80% ethanol, after being photographed with a digital SLR camera equipped with a macro lens to register colour patterns. All crab specimens are deposited in the Crustacea collection of Naturalis Biodiversity Center in Leiden, the Netherlands (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D).

Drawings were made with a stereomicroscope with camera lucida. Carapace lengths and widths were measured to the nearest 0.1 mm using an eyepiece micrometre, with the crabs positioned on a level surface. Abbreviations used: CL, carapace length; CW, carapace width (at widest point); MXP3, third maxilliped; ovig., ovigerous; P, pereopod; G, male gonopod. Carapace measurements are given as CL × CW, in mm.

## Taxonomy

### Cryptochiridae Paul'son, 1875

### *Lithoscaptus* A. Milne-Edwards, 1862

#### *Lithoscaptus semperi* sp. n.

<http://zoobank.org/65F0D837-961A-42B7-8F9E-2C806DD54238>

Figs 1–3

**Type locality.** Tigabu Isl. (06°53'51"N, 117°27'36"E), Kudat, Sabah (N Borneo), Malaysia.

**Coral host holotype.** *Trachyphyllia geoffroyi* (Audouin, 1826).

**DNA barcoding.** A COI sequence (partially, Folmer et al. 1994) of paratype RMNH.Crus.D.54331 has been deposited in GenBank under accession number KP688583.

**Type material. Holotype.** RMNH.Crus.D.56962, ovig. female, 6.4 × 4.6. **Allotype** (with holotype), male, 3.6 × 2.5. Collected by the author from 13 m depth on 8 September 2012. **Paratype.** RMNH.Crus.D.54331, Lubani Rock, Kudat, Sabah (N Borneo), Malaysia (06°53'45.0"N, 117°23'15.8"E), 10–15 m, 07.ix.2012, 1 ovig. female, 6.2 × 4.7, leg. SET van der Meij.

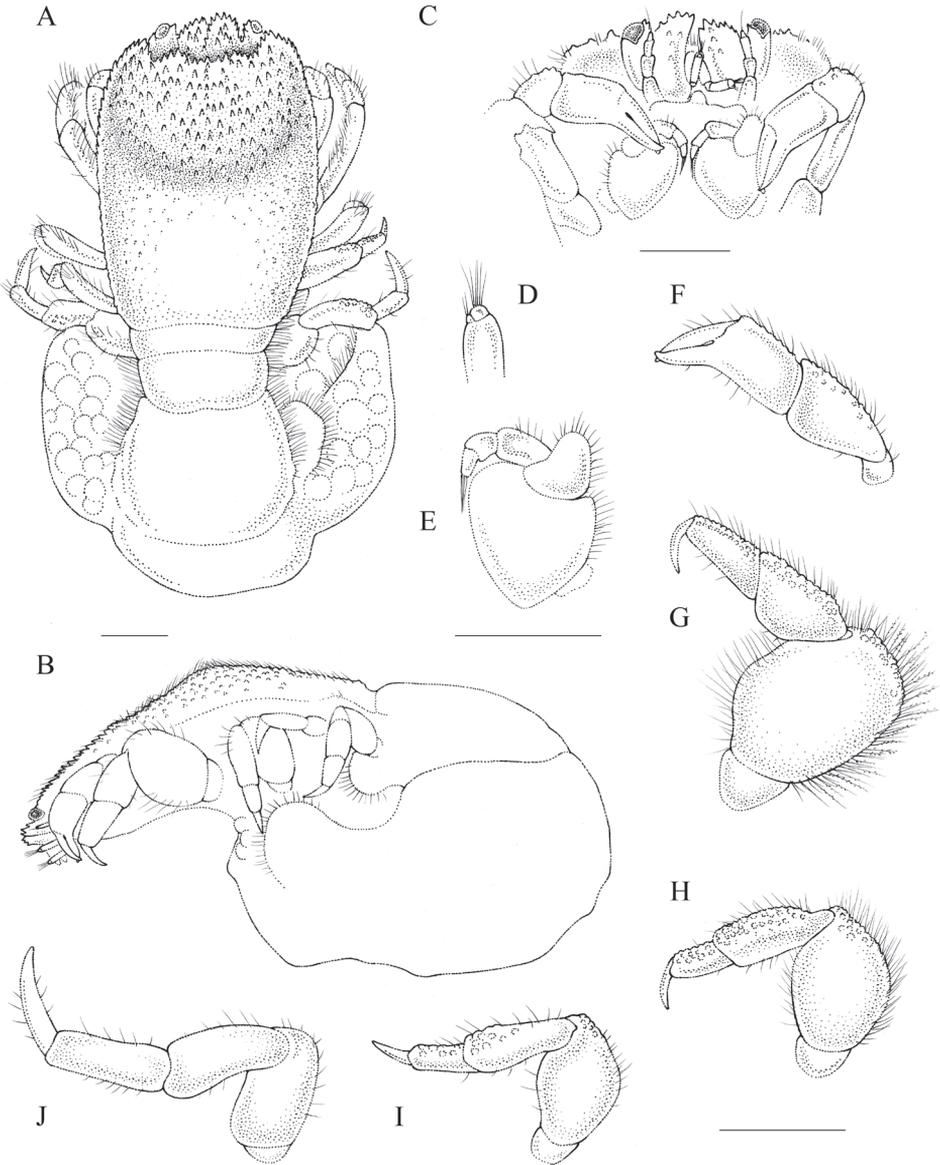
**Material examined. Indonesia:** RMNH.Crus.D.56957, Aer Perang, Lembah Strait (01°28'25"N, 125°14'02"E), ca. 10 m, 02.ii.2012, 1 female, leg. BT Reijnen; RMNH.Crus.D.56958, Tanjung Labuhankompeni, Lembah Strait (01°25'55"N, 125°11'10"E), 28 m, 04.ii.2012, 1 female, leg. BW Hoeksema; RMNH.Crus.D.56959, Kelapadua, Lembah Strait (01°26'19"N, 125°12'49"E), 20 m, 09.ii.2012, 2 juvenile males, leg. BW Hoeksema; RMNH.Crus.D.54250, Tanjung Nanas I, Lembah Strait (01°27'39"N, 125°13'35"E), 25–30 m, 17.ii.201, 1 ovig. female, 1 female, leg. BW Hoeksema; **Malaysia:** RMNH.Crus.D.54259, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E), 10–15 m, 07.ix.2012, 1 ovig. female (slightly damaged), leg. BW Hoeksema; RMNH.Crus.D.54280, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E), 10–15 m, 07.ix.2012, 1 ovig. female, 1 male, leg. BW Hoeksema; RMNH.Crus.D.56960, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E), 10–15 m, 07.ix.2012, 1 male, leg. SET van der Meij; RMNH.Crus.D.56961, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E), 10–15 m, 07.ix.2012, 1 ovig. female, leg. SET van der Meij; RMNH.Crus.D.54312, Tigabu Is., Kudat (06°53'51"N, 117°27'36"E), 9 m, 08.ix.2012, 1 ovig. female (damaged), 1 male, leg. SET van der Meij; RMNH.Crus.D.56963, Fairway Shoal, Kudat (07°07'06"N, 117°30'42"E), 12 m, 10.ix.2012, 1 male, leg. BT Reijnen; RMNH.Crus.D.56964, Belaruan, Kudat (07°01'50"N, 117°00'41"E), ca. 15m, 20.ix.2012, 1 male, leg. BW Hoeksema; RMNH.Crus.D.54258, Tajau, Kudat (06°59'36"N, 116°50'27"E), 21 m, 25.ix.2012, 1 female, 1 male, leg. BW Hoeksema. All material was collected from the scleractinian coral *Trachyphyllia geoffroyi*.

**Description of female holotype.** Carapace (Fig. 1A) rectangular, longer than broad, CL 1.4 times longer than CW; widest near midlength, dorsal surface in lateral view strongly convex in both directions, deflected anteriorly (Fig. 1B); anterior half of carapace with small, sharp tubercles, posterior half smooth with few, rounded granules, cardiointestinal region slightly inflated. Frontal margin armed with small anteriorly directed spines. Frontal margin on ventral side features few, small tubercles. Pterygostomial region fused to carapace.

Eyestalk exposed dorsally, slightly granular, small spines on mesial margin. Cornea anterolateral. Lateral margin of stalk at same level as anterolateral angle; distal margin with small spines (Fig. 1A, C). Distal segment of antennules with protruding article, visible from ventral side (Fig. 1C, D).

Antennular peduncle dorsal surface with small, sharp tubercles, slightly inflated distomesially; apex extending beyond tip of eyestalk; spines on mesial margin larger than those on distal margin. Ventral surface smooth, slightly tapering anteriorly in ventral view (Fig. 1C).

MXP3 (Fig. 1E) exopod rectangular; ischium subtriangular, smooth, mesial and distal margins straight, anteromesial lobe with few simple setae; merus with distolateral



**Figure 1.** Ovigerous female holotype (6.4 × 4.6) of *Lithoscaptus semperi* sp. n. (RMNH.Crus.D.56962) **A** habitus, dorsal view **B** carapace, lateral view **C** anterior margin of carapace, ventral view **D** close-up of antennule **E** MXP3 **F** left P1 (cheliped) **G** left P2 **H** left P3 **I** left P4 **J** left P5. Scale bars 1 mm; A–B, D–E, F–J share scale bars.

projection, simple setae; distal portion of carpus with short, simple setae, dactylus with bundle of setae.

P1 (chelipeds, Fig. 1F) slender; carpus length twice height, scattered small tubercles on dorsal surface, simple setae; propodus length twice height, somewhat granu-

lated, few, scattered setae, fingers slender, mesial surface of fingers smooth, cutting edge entire, tips of fingers crossing.

P2 (Fig. 1G) longer, coarser than P1; ischium without setae; merus stout, plump, smooth with few, small rounded tubercles on distal half of dorsal surface, simple setae on lateral surface, numerous plumose setae on dorsal surface; joint between merus, carpus not extending more than at right angle; carpus smooth with small rounded tubercles on dorsal surface, simple setae on dorsal surface; propodus slightly shorter than carpus, surface smooth with small rounded tubercles on dorsal surface, simple setae on lateral and dorsal surface; dactylus half-length of propodus, smooth, sharp, curved ventrally.

P3 (Fig. 1H) ischium without setae; merus length 1.5 times height, rounded, few rounded tubercles on distal half of dorsal surface, simple setae along dorsal, lateral surface; joint between merus, carpus not extending more than at right angle; carpus length 2.5 times height, rounded tubercles on dorsal surface, simple setae on lateral and dorsal surface; propodus length twice height, rounded tubercles on dorsal surface, scattered simple setae; dactylus similar length as propodus, smooth, sharp, slightly curved ventrally.

P4 (Fig. 1I) similar to P3, less coarse; ischium without setae; merus length 1.5 times height, small rounded tubercles close to joint with carpus, carpus length 2.5 times height, rounded tubercles on distal half of dorsal surface, scattered simple setae; propodus half-length carpus, rounded tubercles on distal half of dorsal surface, few scattered simple setae; dactylus similar length as propodus, smooth, sharp, straight.

P5 (Fig. 1J) ischium without setae; merus, carpus, propodus, dactylus all of equal length, all with short simple setae; carpus, propodus slender compared to merus; dactylus smooth, sharp, slightly curved ventrally.

P3, P4 decreasing in size from P2.

Abdomen enlarged, lateral margins fringed with setae (Fig. 1A, B).

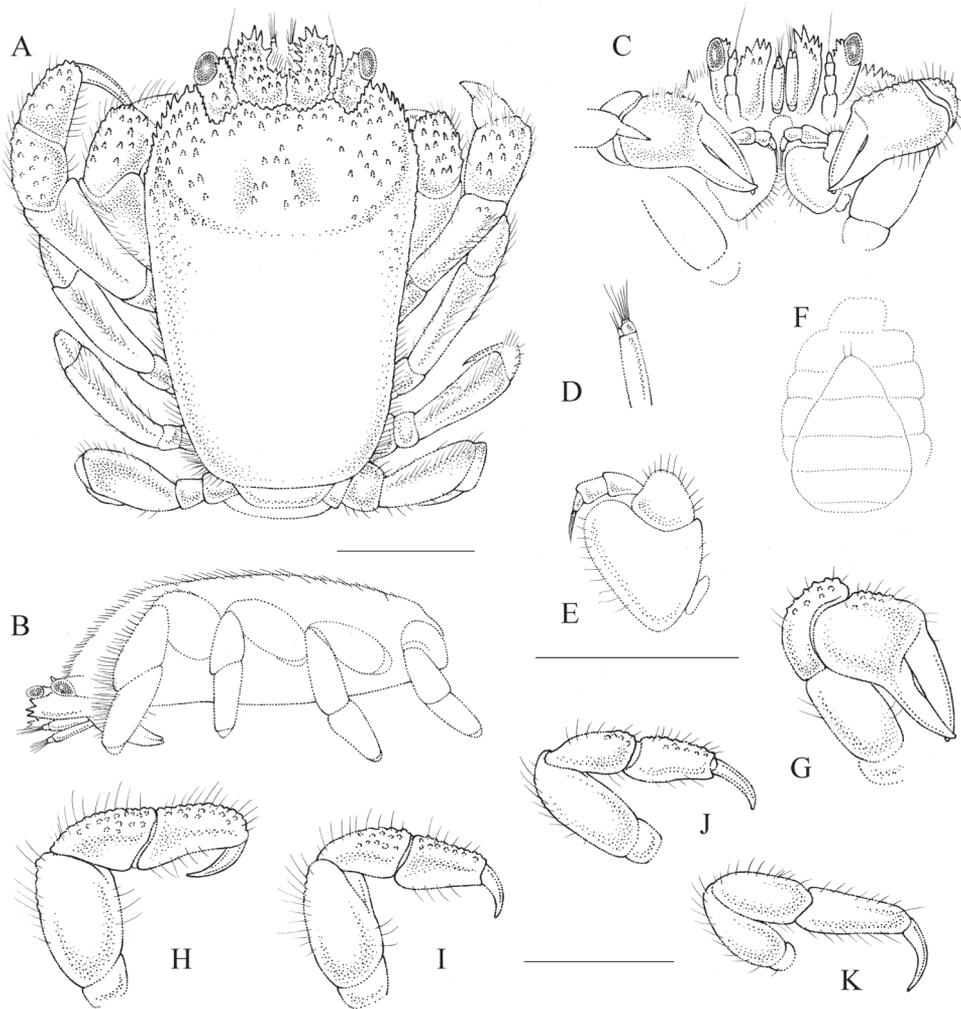
Gonopore (vulva); reniform, size half the height of sternite 6.

**Description of male allotype.** Carapace (Fig. 2A) subrectangular to trapezoid, CL 1.5 times longer than CW, widest near anterior half, convex in lateral view, deflected anteriorly, with broad W-shaped depression (Fig. 2A, B). Anterior half of carapace and carapace margins with small spines, posterior half of carapace smooth.

Ocular peduncles with small spines on distal margin, cornea elliptical, longer than broad; antennal article extending beyond eyestalk, with spines along margins (Fig. 2C). Antennule slender compared to holotype, distal segment of antennules with protruding article (Fig. 2D).

MXP3 (Fig. 2E) exopod rectangular; ischium smooth, triangular, few scattered simple setae on distal and lateral margins, merus with distolateral projection, simple setae; propodus, dactylus of similar length, latter with bundle of short setae.

P1 (chelipeds, Fig. 2G) stout; merus length twice height, smooth; carpus with rounded and conical tubercles, simple setae on dorsal surface; propodus stout, with conical tubercles, simple setae on dorsal surface; fingers slender, mesial surfaces of dactyl slightly gaping, tips of fingers crossing.



**Figure 2.** Male allotype (3.6 × 2.5) of *Lithoscaptus semperi* sp. n. (RMNH.Crus.D.56962) **A** habitus, dorsal view **B** carapace, lateral view **C** anterior margin of carapace, ventral view **D** close-up of antennule **E** MXP3 **F** thoracic sternites **G** right P1 (cheliped) **H** right P2 **I** right P3 **J** right P4 **K** right P5. Scale bar 1 mm; A–C, D–E, F–K share scale bars.

P2 (Fig. 2H) ischium without setae; merus relatively stout, smooth, length twice height, simple short setae on lateral and dorsal surface; carpus, propodus of similar length; carpus with few rounded tubercles and setae on dorsal surface; propodus smooth except for rounded tubercles on dorsal surface, few setae on lateral, dorsal surface, dactylus smooth, sharp, curved ventrally.

P3 and P4 (Fig. 2I, J) similar to P2, somewhat smaller; ischium without setae; merus smooth, simple short setae on lateral and dorsal surface; carpus, propodus of

same length, few rounded tubercles and setae on dorsal surface; dactylus smooth, sharp, curved ventrally.

P5 (Fig. 2K) ischium with few setae; merus, carpus, propodus smooth, with simple short setae on dorsal and lateral surface; dactylus smooth, sharp, curved.

P3, P4 decreasing in size from P2.

Abdomen teardrop-shaped, widest at 4<sup>th</sup> somite; telson slightly pointed with few simple setae (Fig. 2F).

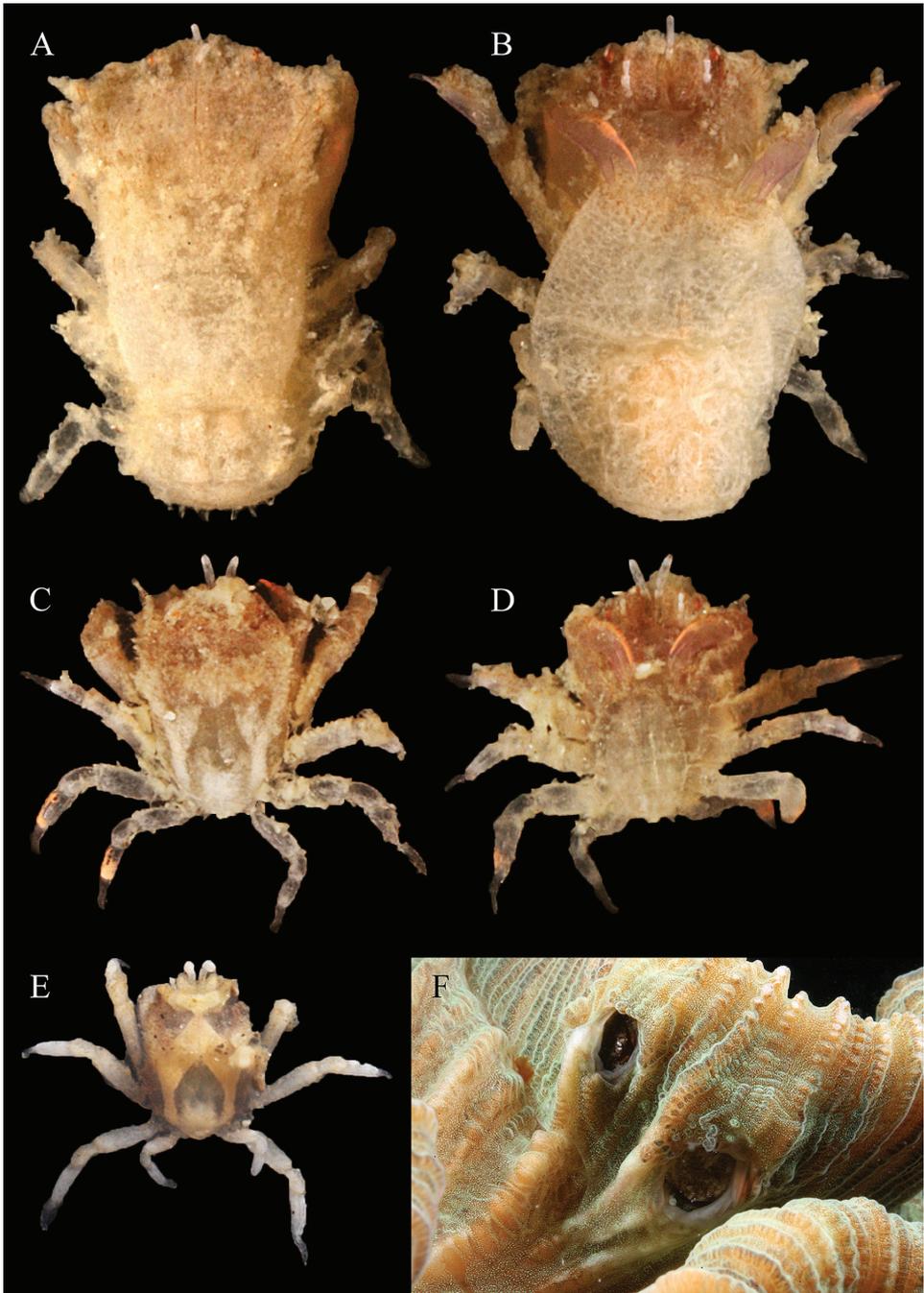
Gonopod 1 almost straight, tapering, apex sharply pointed. Distal margin with 2-3 non-plumose short simple setae, medial margin without setae (examined in RMNH. Crus.D.56964).

**Colour.** Female (Fig. 3A–B): Overall off-white. Pereiopods opaque, carpus, dactylus P1 and P2 translucent violet, sometimes with a pale orange line. Eyes with wide longitudinal brownish-red lines. Male (Fig. 3C–D): Carapace opaque with an off-white distinctive pattern over the whole carapace surface. Pereiopods opaque, P1 carpus, dactylus translucent violet, sometimes with a pale orange line. Eyes brown-red. In juvenile males (Fig. 3E), the carapace pattern is pale orange, pereiopods off-white.

**Placement in genus.** The placement of *Lithoscaptus semperi* sp. n. in the genus *Lithoscaptus* is somewhat tentative. The first (partial) molecular reconstruction of relationships within the Cryptochiridae shows that the genus *Lithoscaptus* is paraphyletic (van der Meij and Reijnen 2014). However, following the diagnosis of *Lithoscaptus* by Kropp (1990), the new species best fits the genus, except for the absence of a proximal tooth on the cutting edge of P1 dactylus and the presence of a distomesial projection of P2 merus in females. Kropp (1994) noted that his new species, *L. prionotus*, had the pterygostomial region not fused to the carapace, unlike other species in the genus. It is likely that the characters defining the genus need to be redefined, or that certain species need to be moved to a new genus.

**Comparisons.** Eight species of *Lithoscaptus* are currently recognised (Ng et al. 2008: 212, Davie 2015). *Lithoscaptus semperi* sp. n. can be distinguished from *L. nami* (Fize & Serène, 1957), *L. tri* (Fize & Serène, 1956) and *L. pardalotus* Kropp, 1995 by not having the internal orbital angle extending beyond the external orbital angle. The new species can be separated from *L. grandis* (Takeda & Tamura, 1983), *L. paradoxus* A. Milne-Edwards, 1862 and *L. prionotus* Kropp, 1994 by the smooth appearance of surface of the carapace, despite the small tubercles on the anterior half of the carapace, and the lack of noticeable spines other than the small spines on the frontal carapace margin. *Lithoscaptus pacificus* (Edmonson, 1933) and *L. helleri* (Fize & Serène, 1957) lack the stout merus with prominent distomesial projection of P2 (female specimens). The off-white carapace colour and translucent violet colour on P1 and P2 in females, and the distinctive carapace pattern in males differs from patterns found on other *Lithoscaptus* species.

**Distribution.** The known distribution of *L. semperi* sp. n. includes northern Borneo and North Sulawesi. Specimens were collected at water depths between 9 and approximately 30 meters. Its host *Trachyphyllia geoffroyi* was described from the Gulf of Suez (Egypt), but this species has a wide distribution that includes the Red Sea, East



**Figure 3.** Colour in life of *Lithoscaptus semperi* sp. n. **A–B** non-ovigerous female (4.5 × 3.2; RMNH. Crus.D.54258) dorsal view and ventral view **C–D** male (2.5 × 1.9; RMNH. Crus.D.54258) dorsal view and ventral view **E** juvenile male (2.0 × 1.6; RMNH. Crus.D.56959) dorsal view **F** in-situ photograph of dwellings (left male, right female) of *L. semperi* sp. n. in *Trachyphyllia geoffroyi* on Lubani Rock reef, Kudat (Malaysia). Photos by BT Reijnen/SET van der Meij.

Africa, Seychelles, Maldives, Nicobar Isls., 'East Indies', China Sea, Philippines, Japan, Australia and New Caledonia (Scheer and Pillai 1983). Based on the widespread distribution of *T. geoffroyi*, a wider distribution range than the two presently recorded locations is expected for *L. semperi* sp. n.

**Coral host.** *Lithoscaptus semperi* sp. n. is so far strictly associated with *T. geoffroyi* (Fig. 3F). It is the first record of associated fauna for this coral host. Colonies of *T. geoffroyi* are free-living, have flabello-meandroid colony shapes and fleshy polyps. Cryptochirids have previously been recorded to inhabit free-living corals; crabs of the genus *Fungicola* are associated with free-living - and attached - mushroom corals (Fungiidae), whereas *Troglocarcinus corallicola* is associated with a wide range of Atlantic corals, including the free-living coral *Manicina areolata* (Mussidae) (Fize and Serène 1957, van der Meij 2014, 2015).

**Remarks.** Fize and Serène (1957: p. 163) report on *Cryptochirus coralliodytes* from *Trachyphyllia* based on a record of Semper (1881: p. 221) who writes: "I found them [C. coralliodytes] in the Philippine Archipelago in cavities in *Goniastrea Bournoni* [= *Goniastrea retiformis* (de Lamarck, 1816)], in an undetermined true *Astræa*, which was unfortunately lost, also in an undescribed *Trachyphyllia*; finally I received a new form through A. Agassiz from the West Indian seas, which may perhaps form a distinct genus, though it is very nearly allied to the first. It also lives in a *Trachyphyllia*." The coral genus *Trachyphyllia* is described from the Red Sea and has a widespread Indo-Pacific distribution; however, it does not occur in the Atlantic Ocean. The most similar Atlantic species would be *Manicina areolata* (Linnaeus, 1758). Furthermore, on p. 453 (note 103 belonging to p. 221) Semper writes: "This crab, living in *Trachyphyllia*, a West Indian coral, is extremely like *Cryptochirus*, and perhaps belongs to the same genus; this can only be determined by future and more exact examination. But the 'cave dwelling' of this West Indian crab is perfectly unlike that of the Eastern species, which is found from the Red Sea as far as the Pacific Ocean; it is not cylindrical, but has one side quite flat, so that its transverse section is almost exactly a half-circle; the underside of the crab rests against the flat side of the cavity." The gall crab *Troglocarcinus corallicola* Verrill, 1908 has been recorded from a wide range of hosts, including *M. areolata* (Kropp and Manning 1987, van der Meij 2014). As mentioned by Semper (1881), the dwelling of *T. corallicola* in *M. areolata* is shaped like a half-circle (see e.g. Van der Meij 2014: Fig. 1B); therefore, it seems plausible that Semper was referring to the coral *M. areolata* when he discussed a West Indian *Trachyphyllia*. Alternatively, Semper could have been referring to the Atlantic genus *Colpophyllia* because Milne Edwards and Haime (1849), who established *Trachyphyllia*, compared their new genus with *Colpophyllia* (see Huang et al. [2014] for a discussion on the genus *Trachyphyllia*). Like *M. areolata*, *Colpophyllia natans* (Houttuyn, 1772) also hosts *T. corallicola* (see van der Meij 2014). It remains unclear whether Semper found gall crabs in Indo-Pacific corals currently recognized as *Trachyphyllia geoffroyi*. Semper is not known to have formally described any gall crab species (Ng et al. 2008).

**Etymology.** Named after the German naturalist Carl Gottfried Semper (1832–1893), who was the first to mention gall crabs occurring in *Trachyphyllia*.

## Acknowledgements

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# A remarkable new species of *Alloscorpiops* Vachon, 1980 from a cave in Vietnam (Scorpiones, Euscorpiidae, Scorpiopinae)

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## Abstract

Among the genera of the subfamily Scorpiopinae Kraepelin, 1905 *Alloscorpiops* remains rather discrete. Only recently new species were added to this genus, increasing its number from two to five. Therefore, species of *Alloscorpiops* remain rare. One remarkable new species, *Alloscorpiops troglodytes* **sp. n.**, is described on the basis of a single male specimen collected inside a cave from Song Thanh Nature Reserve, Cha Vanh Commune, Nam Giang District in Vietnam. The new species presents most features exhibited by scorpions of the genus *Alloscorpiops*, but it is characterized by reduced size, slender body and elongated pedipalps. This new scorpion taxon represents the third species of Scorpiopinae discovered in a cave system, and may be another endemic element in the fauna of Vietnam.

## Keywords

Scorpion, Scorpiopinae, *Alloscorpiops*, new species, Vietnam

## Introduction

In his revision of the genus *Scorpiops*, Vachon (1980) described three new subgenera, *Alloscorpiops*, *Euscorpiops*, and *Neoscorpiops*, in addition to the nominotypical subgenus *Scorpiops*. *Alloscorpiops* was defined on the basis of an important ‘majorante’ neobothriotaxy with 10–12 ventral trichobothria on the surface of pedipalp chela-hand, whereas the other subgenera presented only four trichobothria. Vachon (1980) assigned two species to this subgenus: *Scorpiops (Alloscorpiops) anthracinus* Simon, 1887 (as type species of the subgenus) and *Scorpiops (Alloscorpiops) lindstroemii* Thorell, 1889.

Stockwell (1989), in an unpublished thesis dissertation, proposed raising all the subgenera within the family Scorpiopidae to the rank of genera; however, his proposition could not be validated since his dissertation was never published. Finally, Lourenço (1998) confirmed this decision. The four subgenera were elevated to generic rank and the monotypic genera *Parascorpiops* Banks, 1928 and *Dasyscorpiops* Vachon, 1974 were added, thus bringing the total number of genera to six.

In the present note, a remarkable new species belonging to the genus *Alloscorpiops* is described from a cave in Song Thanh Nature Reserve, Cha Vanh Commune, Nam Giang District in Central Vietnam. This new scorpion taxon is the third species of Scorpiopinae (Lourenço and Pham 2013, 2014) to be discovered in a cave system and the first one belonging to the genus *Alloscorpiops*. It may be yet another endemic element in the fauna of this country.

## Present composition of the genus *Alloscorpiops* Vachon, 1980

*Alloscorpiops (Alloscorpiops) anthracinus* (Simon, 1887), Myanmar

*Alloscorpiops (Alloscorpiops) lindstroemii* (Thorell, 1889), Myanmar

*Alloscorpiops (Laoscorpiops) calmonti* Lourenço, 2013, Laos

*Alloscorpiops (Alloscorpiops) citadelle* Kovařík, 2013, Thailand

*Alloscorpiops (Alloscorpiops) wongpromi* Kovařík, Soleglad & Košulič, 2013, Laos, Thailand

*Alloscorpiops (Alloscorpiops) troglodytes* sp. n., Vietnam

The species *Alloscorpiops lindstroemii* (Thorell, 1889) was considered a synonym of *Alloscorpiops anthracinus* (Simon, 1887) by Kovařík (2013). This decision is, as usual, sustained mainly by personal speculation without the examination of the type material of both species (see also Lourenço 2013; Kovařík et al. 2013; Lourenço and Pham 2015). Inversely, Vachon (1980; in litt.) did examine the types of these species and found some differences which led him to consider them as valid. Some of these characters are expressed herein (key presented after the description). In absence of more solid evidence to validate this synonymy, *Alloscorpiops lindstroemii* (Thorell, 1889) is restored at present.

## Methods

Illustrations and measurements were produced using a Wild M5 stereo-microscope with a drawing tube and an ocular micrometer. Measurements follow Stahnke (1970) and are given in mm. Trichobothrial notations follow Vachon (1974, 1980) and morphological terminology mostly follows Vachon (1952) and Hjelle (1990).

## Taxonomic treatment

Family Euscorpiidae Laurie, 1896

Subfamily Scorpiopinae Kraepelin, 1905

Genus *Alloscorpiops* Vachon, 1980

**Diagnosis of the new species.** The new species shows several of the characteristics already defined for the genus *Alloscorpiops* (Vachon 1980; Soleglad and Sissom 2001). It presents, however, a small size relative to other species of the genus, male 20.9 mm in total length and a very pale yellow coloration. The new species is characterized by the trichobothrial patterns of some 'territories' or series. Femur with three trichobothria: dorsal, internal and external. Patella with two dorsal, one internal, 14 ventral and only 21 external trichobothria. Chela-hand with an unusual number of 9 ventral trichobothria, two dorsal (**Dt**, **Db**), two internal (**ib**, **it**), **Est**, five **Et**, **Esb** and three trichobothria in the **Eb** series. The annular ring is very weakly marked. Pectines with 9-9 teeth and absence of fulcra.

## Description of the new species

*Alloscorpiops (Alloscorpiops) troglodytes* sp. n.

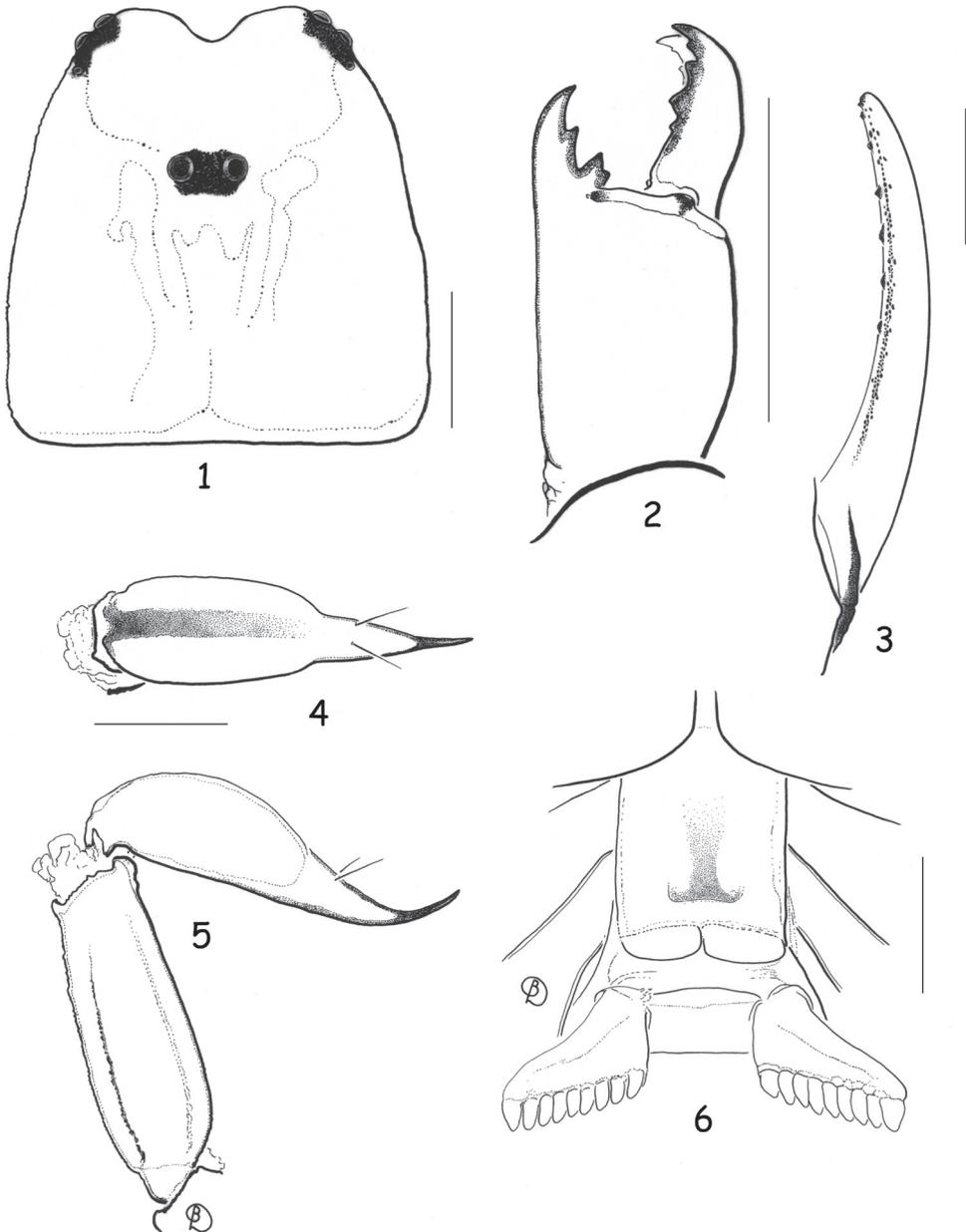
<http://zoobank.org/299E145C-F085-4012-B8D4-B66EBBF7A616>

Figs 1–13

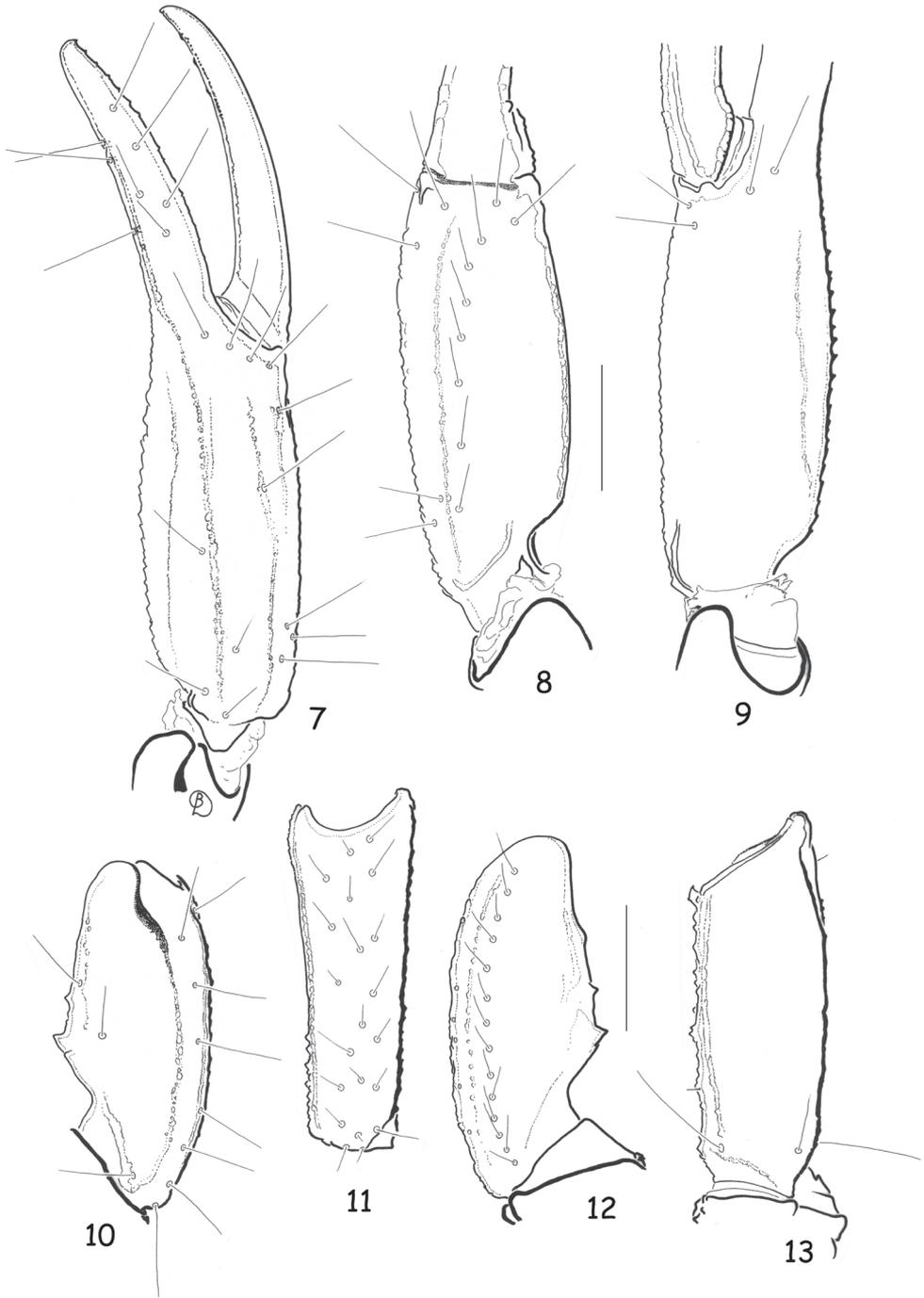
**Type materials.** Vietnam, Song Thanh Nature Reserve, Cha Vanh Commune, Nam Giang District, inside cave (Fig. 14), approximately 60 m from entry, 20/XII/1958 (B. Dejenböl). Male Holotype. Deposited in the Muséum national d'Histoire naturelle, Paris.

**Etymology.** The specific name refers to the natural habitat where the new species was found.

**Description.** The general coloration is yellow to pale yellow. Carapace and tergites yellow. Metasomal segments yellow to pale yellow; telson yellow; base of aculeus yellow and tip slightly reddish. Chelicerae yellow without spots; teeth slightly reddish. Pedipalps yellow; chela fingers slightly red. Legs pale yellow. Venter pale yellow; pectines totally pale, almost white.



**Figures 1–6.** *Alloscorpiops troglodytes* sp. n. Male holotype. **1** Carapace **2** Chelicera, dorsal aspect **3** Cutting edge of movable finger with rows of granules **4** Telson, ventral aspect **5** Metasomal segment V and telson, lateral aspect **6** Ventral aspect, showing sternum, genital operculum, and pectines. Scale bars: 1 mm.



**Figures 7–13.** *Alloscorpions troglodytes* sp. n. Male holotype. Trichobothrial pattern **7–9** Chela, dorso-external, ventral and internal aspects **10–12** Patella, dorsal, external and ventral aspects **13** Femur, dorsal aspect. Scale bars: 1 mm.



**Figure 14.** A typical cave of the Song Thanh Nature Reserve cave system. View of the entrance. [Photo courtesy of N.Q. Truong]

*Morphology.* Carapace weakly granular, furrows moderately deep. Median eyes anterior to centre of carapace; three pairs of lateral eyes, the third pair only slightly smaller than the first two. Sternum pentagonal, longer than wide. Tergites weakly granulated, almost smooth; VII with four weakly marked carinae. Pectinal tooth count 9-9; fulcra absent. Sternites smooth and shiny; VII with four vestigial carinae and some punctations. Metasomal segment I wider than long; segment II as long as wide; segments III to V longer than wide; 10-8-8-8-7 carinae present on segments I to V, weakly marked; dorsal carinae on segments I–IV with a single, weakly marked posterior spinoid granule; metasomal tegument very weakly granulated almost smooth; ventral carina on segment V without spinoid granules. Telson vesicle totally smooth. Pedipalps: femur with dorsal internal, dorsal external, ventral internal and ventral external carinae moderately marked; tegument weakly granular. Patella with dorsal internal, ventral internal, dorsal external, ventral external and external carinae moderately marked; two/three inconspicuous spinoid granules present on internal aspect, the interno-ventral being slightly larger than the interno-dorsal granule; tegument weakly granular. Chela with dorsal marginal, external secondary, ventral internal and ventral carinae moderately to strongly marked; other carinae moderately to weakly marked; tegument granulated dorsally and ventrally. Chelal fingers with two longitudinal series of granules, almost fused, and a



**Figure 15.** Map of southeast Asia showing the known distribution of the species belonging to the genus *Alloscorpiops*: *Alloscorpiops anthracinus* (1), *Alloscorpiops lindstroemii* (2), *Alloscorpiops calmonti* (3), *Alloscorpiops citadelle* (4), *Alloscorpiops wongpromi* (5) and *Alloscorpiops troglodytes* sp. n. (6).

few inner accessory granules. Chelicerae dentition as in figure 2 (Vachon 1963); five/six teeth on ventro-internal face of movable finger. Trichobothriotaxy type **C**, as in figures 7–13 (Vachon 1974): see diagnosis.

*Morphometric values (in mm) of male holotype.* Total length (including telson) 20.9. Carapace: length 3.2; anterior width 2.1; posterior width 3.3. Mesosoma length 7.4. Metasomal segment I: length 0.9, width 1.2; II: length 1.1, width 1.1; III: length 1.2, width 1.0; IV: length 1.4, width 0.9; V: length 2.3, width 0.8, depth 0.7. Telson



**Figure 16.** Natural habitat of the new species, *Alloscorpiops troglodytes* sp. n., covered by evergreen forests in Song Thanh Nature Reserve region in Central Vietnam. [Photo courtesy of N.Q. Truong]

length 2.9. Vesicle: width 0.8, depth 0.8. Pedipalp: femur length 3.1, width 1.2; patella length 2.7, width 1.3; chela length 6.1, width 1.3, depth 1.2; movable finger length 3.1.

**Simplified key to the species of *Alloscorpiops***

- 1 Chela of pedipalp with 3 trichobothria on the **Eb** series.....2
- Chela of pedipalp with 5 trichobothria on the **Eb** series.....  
.....*Alloscorpiops (Laoscorpiops) calmonti*
- 2 Chela of pedipalp with 10 to 13 ventral trichobothria; patella with 15 to 22 ventral trichobothria .....3
- Chela of pedipalp with 9 ventral trichobothria; patella with 14 ventral trichobothria.....*Alloscorpiops troglodytes* sp. n.
- 3 Patella of pedipalp with 15–16 ventral and 23–25 external trichobothria ...4
- Patella of pedipalp with 19–21 ventral and 29–37 external trichobothria ...5
- 4 Patella of pedipalp with 16 ventral and 23 external trichobothria .....  
.....*Alloscorpiops anthracinus*
- Patella of pedipalp with 15 ventral and 25 external trichobothria .....  
.....*Alloscorpiops lindstroemii*

- 5 Patella of pedipalp with 19-21 ventral and 29-34 external trichobothria.....  
 ..... *Alloscorpiops citadelle*
- Patella of pedipalp with 21-22 ventral and 33-37 external trichobothria.....  
 ..... *Alloscorpiops wongpromi*

### Ecological aspects of Nam Giang district and Song Thanh Nature Reserve

Cha Vanh commune is located in Nam Giang District, within Song Thanh Nature Reserve in Quang Nam Province along the Vietnam/Laos border. Nam Giang has one of the largest areas of tropical forest in Vietnam and is situated at the intersection of several biogeographical sectors (Fig. 15).

Nam Giang is located within coordinates 15°13' to 15°41' N and 107°21' to 107°50' E. In the north, it borders highway 14D, which runs from east to west between Thanh My and Dak Oc, along the Vietnam and Laos border. In the south, it connects with Kontum Province at the crest of Lo Xo Mountain and the highway 14D. To the west, it is bordered by Laos PDR and to the east by the waterways of the Thanh and Cai rivers. The altitude of the area ranges from 80 to 2,032 metres above sea level (m.a.s.l.).

This region is part of the central coastal climate zone. With an average temperature of 24.6 °C and a minimum temperature of 20 °C, the weather is hot in comparison with northern Vietnam. The rainy season in the area arrives two to three months after the rainy season north of the Truong Son Mountain range. It ranges from August to December/January, with the most intensive rainfall season occurring between September and November. The dry season coincides with a hot, dry western wind, which speeds up the evaporation process, reduces the humidity, and has a negative impact on the floristic composition of the forest.

There are three main soil types in the area: ferralite humus on rocky mountains (49.7 per cent), typical ferralite in low hill areas (48.9 per cent) and alluvial soils in valleys.

The area of Nam Giang is an important component of the Priority Central Truong Son landscape of the Truong Son ecosystem. A rich biodiversity and high numbers of endemic species makes Nam Giang one of the high-priority biodiversity areas in Vietnam. The fauna and flora of Nam Giang is diverse and 95% of Nam Giang is covered by evergreen forests (Fig. 16). Nevertheless the total inventory work on the biodiversity of this region is far from being complete, and many new taxa can be expected to be found during future inventories.

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# Two new species of the *Liolaemus elongatus-kriegi* complex (Iguania, Liolaemidae) from Andean highlands of southern Chile

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## Abstract

The *elongatus-kriegi* complex is one of the most diverse clades of the *Liolaemus (sensu stricto)* subgenus of lizards. There are currently 29 species recognized in this group distributed between Chile and Argentina. Based on molecular evidence, there seem to be five main clades nested within this complex: the *elongatus*, *leopardinus*, *kriegi*, *petrophilus* and *punmahuida* clades. *Liolaemus buergeri* and *L. kriegi*, both of the *kriegi* clade, were believed to inhabit the surroundings of the Laja Lagoon, in the Biobío Region of Chile. Moreover, this Chilean population of *L. kriegi* was recently recognized as an undescribed taxon called “*Liolaemus* sp. A” based on molecular phylogenetics. In this work, we studied these two populations of the Laja Lagoon and provided the morphological diagnosis to describe them as two new species: *L. scorialis* sp. n. and *L. zabalai* sp. n., previously considered *L. buergeri* and “*L. kriegi* *Liolaemus* sp. A” respectively. Additionally, we identified another population of *L. scorialis* in the vicinity of La Mula Lagoon in the Araucanía Region of Chile. *Liolaemus scorialis* differs from almost all of the species of the *elongatus-kriegi* complex by its considerably smaller size. Nevertheless, without molecular data we cannot assign it to any particular subclade. *Liolaemus zabalai* belongs to the *kriegi* clade based on published molecular phylogenies. Finally, we provide some natural history data on both species and we document for the first time the presence of *L. neuquensis* in Chile from a museum specimen from La Mula Lagoon.

## Keywords

*Liolaemus buergeri*, *Liolaemus kriegi*, new species, lizard, Laja Lagoon, Biobío

## Introduction

*Liolaemus* is a diverse genus of South American lizards, with currently 245 species (Uetz and Hošek 2014) grouped into two subgenera: *Liolaemus (sensu stricto)* and *Eulaemus* (e.g. Laurent 1985, Schulte et al. 2000). Each of these subgenera has been divided into several groups based on phylogenetic relationships (Abdala 2007, Avila et al. 2006, Fontanella et al. 2012, Lobo 2005).

The *elongatus-kriegi* complex Cei (1979), is one of the most diverse groups of the *Liolaemus (sensu stricto)* subgenus with currently 29 species distributed in Chile and Argentina. In a phylogenetic study based on three mitochondrial genes, Morando et al. (2003) found that this complex is subdivided into three clades: *elongatus*, *kriegi* and *petrophilus*. Later, Avila et al. (2010a) based on one mitochondrial locus, added a fourth clade: the *punmahuida* clade. Finally, Esquerré et al. (2014) added a fifth clade comprising only Chilean endemic species: the *leopardinus* clade. An alternative classification has been proposed by Lobo (2005) and updated by Lobo et al. (2010b), based mainly on morphological and lifestyle traits, which classifies these species in three groups: *elongatus* (which includes the *capillitas* subgroup), *kriegi* and *leopardinus*, with a different arrangement compared with the molecular hypothesis (Table 1 and Table 2).

Currently, the *elongatus-kriegi* complex (Avila et al. 2012, Esquerré et al. 2014, Morando et al. 2003) or *elongatus*, *kriegi* and *leopardinus* groups (Lobo 2005, Lobo et al. 2010b) includes the following species: *Liolaemus antumalguen* Avila et al., 2010, *L. austromendocinus* Cei, 1974, *L. buergeri* Werner 1907, *L. burmeisteri* Avila et al. 2012, *L. carlosgarini* Esquerré et al. 2013, *L. capillitas* Hulse, 1979, *L. choique* Abdala et al. 2010, *L. cristiani* Núñez et al. 1991, *L. dicktracyi* Espinoza & Lobo, 2003, *L. elongatus* Koslowsky, 1896, *L. flavipiceus* Cei & Videla, 2003, *L. frassinettii* Núñez, 2007, *L. gununakuna* Avila et al. 2004, *L. heliodermis* Espinoza et al. 2000, *L. kriegi* Müller & Hellmich, 1939, *L. leopardinus* Müller & Hellmich, 1932, *L. parvus* Quinteros et al. 2008, *L. petrophilus* Donoso-Barros & Cei, 1971, *L. punmahuida* Avila et al. 2003, *L. ramonensis* Müller & Hellmich, 1932, *L. shitan* Abdala et al. 2010, *L. smaug* Abdala et al. 2010, *L. talampaya* Avila et al. 2004, *L. thermarum* Videla & Cei, 1996, *L. tregenzai* Pincheira-Donoso & Scolaro, 2007, *L. tulkas* Quinteros et al. 2008, *L. ubaghsi* Esquerré et al. 2014, *L. umbrifer* Espinoza & Lobo, 2003 and *L. valdesianus* Hellmich, 1950.

*Liolaemus buergeri*, of the *kriegi* clade, was described from El Planchón Volcano, Maule Region, Chile (Werner 1907). This species has been traditionally believed to be widely distributed in Chile and Argentina (Cei 1986, Pincheira-Donoso 2001). However, its current wide distribution is in part due to cases of misidentification and a lumping of cryptic species (Medina et al. 2013). Donoso-Barros (1970) extended the southern distribution of *L. buergeri* to the Andes of Talca, Maule Region, Chile (50 km S from El Planchón Volcano).

**Table 1.** Species of the *elongatus-kriegi* complex grouped by clades, based on mitochondrial molecular phylogenies. (1) Species included by Morando et al. (2003). (2) Species added by Avila et al. (2004). (3) Species added by Avila et al. (2010a). (4) Species added by Avila et al. (2012). (5) Species added fide Esquerré et al. (2014). *Liolaemus thermanum* is included in the *elongatus* clade by Avila et al. (2010a) but omitted by Avila et al. (2012).

<i>elongatus</i> clade	<i>kriegi</i> clade	<i>leopardinus</i> clade	<i>petrophilus</i> clade	<i>punmahuida</i> clade
<i>L. antumalguen</i> (3)	<i>L. buergeri</i> (1)	<i>L. frassinettii</i> (5)	<i>L. austromendocinus</i> (1)	<i>L. flavipiceus</i> (3)
<i>L. burmeisteri</i> (4)	<i>L. kriegi</i> (1)	<i>L. leopardinus</i> (5)	<i>L. capillitas</i> (1)	<i>L. punmahuida</i> (3)
<i>L. elongatus</i> (1)		<i>L. ramonensis</i> (5)	<i>L. dicktracyi</i> (2)	
<i>L. smaug</i> (4)		<i>L. ubaghsi</i> (5)	<i>L. gununakuna</i> (2)	
<i>L. thermanum</i> (3)		<i>L. valdesianus</i> (5)	<i>L. parvus</i> (3)	
			<i>L. petrophilus</i> (1)	
			<i>L. talampaya</i> (2)	
			<i>L. tulkas</i> (3)	
			<i>L. umbriifer</i> (2)	

**Table 2.** Species of the *elongatus-kriegi* complex by groups, based on morphological, skeletal and lifestyle traits phylogeny according to (1) Lobo (2005), (2) updated by Lobo et al. (2010b) and (3) fide Esquerré et al. (2013). The *capillitas* subgroup is nested into *elongatus* group (Lobo et al. 2010b).

<i>capillitas</i> subgroup	<i>elongatus</i> group	<i>kriegi</i> group	<i>leopardinus</i> group
<i>L. capillitas</i> (1)	<i>L. austromendocinus</i> (2)	<i>L. buergeri</i> (1)	<i>L. frassinettii</i> (2)
<i>L. dicktracyi</i> (1)	<i>L. carlosgarini</i> (3)	<i>L. cristiani</i> (1)	<i>L. leopardinus</i> (1)
<i>L. heliodermis</i> (1)	<i>L. elongatus</i> (1)	<i>L. kriegi</i> (1)	<i>L. ramonensis</i> (1)
<i>L. talampaya</i> (2)	<i>L. flavipiceus</i> (2)		<i>L. valdesianus</i> (1)
<i>L. tulkas</i> (2)	<i>L. gununakuna</i> (2)		
<i>L. umbriifer</i> (1)	<i>L. parvus</i> (2)		
	<i>L. petrophilus</i> (2)		
	<i>L. punmahuida</i> (2)		
	<i>L. thermanum</i> (2)		
	<i>L. tregenzai</i> (2)		

Later, Pincheira-Donoso (2001) extended the Chilean southern distribution of *L. buergeri* to the Batea-Mahuida Volcano (Araucanía Region, 240 km S from El Planchón Volcano) and pointed out that he also examined three specimens from the Laja Lagoon (Biobío Region, Chile, 150 km S from El Planchón Volcano); but Pincheira-Donoso and Núñez (2005) indicated that the specimens from Batea-Mahuida Volcano indeed correspond to *L. elongatus*, whereas the status of “*L. buergeri*” from the Laja Lagoon in Chile remains uncertain. In regards to Argentina, Cei (1986) stated that this species occurs in Mendoza and Neuquén Provinces, but Morando et al. (2003) and Medina et al. (2013), based on genetic and morphological evidence, respectively, indicated that several Argentinean populations attributed to *L. buergeri* correspond to at least three undescribed species.

*Liolaemus kriegi*, also of the *kriegi* clade, was described from Estancia El Cóndor, Río Negro Province, Argentina (Müller and Hellmich 1939a). Later, Donoso-

Barros (1966) extended its northern distribution to the Cordillera de Curicó, Maule Region, Chile, 650 km N of Estancia El Cóndor; and to the Laja Lagoon, Biobío Region, Chile, 400 km N of Estancia El Cóndor (Donoso-Barros 1974). Morando et al. (2003), based on mitochondrial genes, found three candidate species related to *L. kriegi*, all from Argentina and previously assigned to *L. buergeri*: *Liolaemus* sp. A (from Caviahue, Neuquén Province), *Liolaemus* sp. B (from Ranquil Norte, Neuquén Province) and *Liolaemus* sp. C (from Laguna Los Barros, Neuquén Province). Medina et al. (2013), in a morphological analysis of these populations, corroborated the status of candidate species of these *Liolaemus* sp., adding new localities for *Liolaemus* sp. A, including samples from the Laja Lagoon (Chile) which corresponds to the species previously identified as *L. kriegi* by Donoso-Barros (1974). Also, Medina et al. (2013) found another candidate species from Argentina (*Liolaemus* sp. D), previously identified as *L. buergeri* by Morando et al. (2003). Recently, Medina et al. (2014) in a new phylogenetic study based on mitochondrial and nuclear genes, corroborate the previous studies and provide strong evidence for *Liolaemus* sp. A as a candidate species, also based on samples from Chile (Laja Lagoon) and Argentina (several localities of Neuquén Province).

Here, we studied the taxonomic status of the southernmost currently-recognized Chilean population of “*Liolaemus buergeri*”, from the vicinity of the Laja Lagoon, Biobío Region; and of “*L. kriegi*/*Liolaemus* sp. A” from the same locality. This population of “*L. buergeri*” is described as a new species which differs greatly from *L. buergeri* and almost all species of the *elongatus-kriegi* complex by its small snout-vent length (less than 70.0 mm). Additionally, specimens of this new species are recorded from La Mula Lagoon, Araucanía Region, Chile. For “*L. kriegi*/*Liolaemus* sp. A”, we provide a full description and diagnosis of this new species belonging to the *kriegi* clade.

## Materials and methods

We examined specimens of almost all Chilean species currently considered as belonging to the *Liolaemus elongatus-kriegi* complex. The morphological characters were examined according to Etheridge (1995), Lobo (2005), Abdala et al. (2010) and Avila et al. (2010a, 2012). Body measurements were taken with a digital vernier caliper (0.02 mm precision). Measurements are provided as mean  $\pm$  standard deviation ( $\bar{x} \pm SD$ ). The Mann–Whitney U test was used to compare the new species and some related species. Scales were observed with different magnifying lenses and scalation and measurements were recorded on the right side of the specimen, unless otherwise indicated. Dorsal scales were counted between the occiput and the level of the anterior border of the hind limbs. Ventral scales were counted from mental scale to the anterior margin of cloacal opening. Stomach and intestinal contents were observed under a binocular microscope for one specimen of each new species. The specimens examined are listed in Appendix 1. Data for Argentinean species were taken from the literature. *Liolaemus ceii* is not accepted as valid species in this work (see discussion). Museum codes are as

follow: **MRC** (Museo Regional de Historia Natural, Concepción), **MZUC** (Museo de Zoología, Universidad de Concepción) and **SSUC** (Colección de Flora y Fauna Patricio Sánchez Reyes, Pontificia Universidad Católica de Chile).

## Results

### *Liolaemus scorialis* sp. n.

<http://zoobank.org/35B1E4BC-4EA1-4FEF-B025-B93D5C5A9CB9>

Fig. 1

*Liolaemus buergeri* (in part?), Pincheira-Donoso, 2001. Not. Mens. Mus. Nac. Hist. Nat., Chile, 346: 8.

*Liolaemus buergeri* (in part?), Pincheira-Donoso & Núñez, 2005. Pub. Oc. Mus. Nac. Hist. Nat., Chile, 59: 285.

**Holotype.** SSUC Re 617 (Fig. 1). Male collected 7 km NW of the summit of the Antuco Volcano, near the Laja Lagoon, Biobío Region, Chile (37°21'S – 71°23'W, 1450 m). Collected by J. Troncoso-Palacios, F. Urra and H. Díaz. 08/01/2014.

**Paratypes.** SSUC Re 615–16 two males and 612–614 three females (Figs 1 and 3). The same data as the holotype. MRC 675, 677, 680, 682. Four males. La Mula Lagoon (37°53'S – 71°22'W), Ralco National Reserve. Unknown coll. 01/12/2001.

**Etymology.** The species name refers to the habitat, which is composed of accumulations of igneous rocks from the Antuco Volcano, called “scoria” from the Greek “skoria”. We propose the common name “Slag Lizard” in English and “Lagarto del escorial” in Spanish.

**Diagnosis.** *Liolaemus scorialis* belongs to the *elongatus-kriegi* complex, but its specific assignation to a particular subclade is currently unknown since we have no molecular data for this new species, and molecular and morphological phylogenies for the *elongatus-kriegi* complex disagree in the arrangement of this complex subgroups (see discussion).

Below a wide diagnosis is provided on aspect of all species of the complex. *Liolaemus scorialis* differs from almost all species of the *elongatus-kriegi* complex by its size (maximum SVL = 69.9 mm), smaller than *L. antumalguen* (Table 3), *L. austromendocinus* (max. SVL = 103.0 mm, Espinoza et al. 2000), *L. buergeri* (Table 3, Fig. 2), *L. burmeisteri* (Table 3), *L. capillitas* (max. SVL = 93.0 mm, Espinoza et al. 2000), *L. choique* (Table 3), *L. dicktracyi* (max. SVL = 91.0 mm, Espinoza and Lobo 2003), *L. elongatus* (max. SVL = 94.7 mm, Avila et al. 2012), *L. flavipiceus* (Table 3, Fig. 2), *L. frassinettii* (max. SVL = 91.1 mm), *L. gununakuna* (max. SVL = 97.5 mm, Avila et al. 2004), *L. kriegi* (max. SVL = 101.0 mm; Avila et al. 2003), *L. leopardinus* (max. SVL = 98.2 mm), *L. petrophilus* (max. SVL = 100.0 mm; Espinoza et al. 2000), *L. punmahuida* (Table 3), *L. ramonensis* (max. SVL = 94.9 mm), *L. shitan* (max. SVL = 98.3 mm, Abdala et al. 2010), *L. talampaya* (max. SVL = 85.5 mm, Avila et al. 2004), *L. thermarum* (max. SVL = 85.0 mm, Videla and Cei 1996), *L. tregenzai* (Table 3),



**Figure 1.** *Liolaemus scorialis* sp. n. **A, B** Holotype, male **C, D** Paratype, female **E** Paratype, male **F** Paratype, female. All from the type locality, 7 km NW of the summit of the Antuco Volcano, near the Laja Lagoon, Biobío Region, Chile.

*L. ubaghsi* (max. SVL = 89.6 mm), *L. umbrifer* (max. SVL = 89.0 mm, Espinoza and Lobo 2003), *L. valdesianus* (max. SVL = 93.4 mm) and “*L. kriegel*/*Liolaemus* sp. A” (max. SVL = 92.0 mm, described below).

*Liolaemus scorialis* has probably been previously confused with *L. buergeri* (see discussion), but in addition to the size difference, *L. scorialis* differs from *L. buergeri* because the latter has a vertebral stripe on the tail, whereas the tail is ringed in *L. scorialis*. Moreover, *L. buergeri* has more midbody scales ( $x = 89.4 \pm 5.5$ ,  $n = 14$ ) than *L. scorialis* ( $x = 82.0 \pm 4.7$ ,  $n = 10$ ) (Mann–Whitney  $U = 20.5$ ,  $P < 0.01$ ,  $DF = 21$ ) and more dorsal scales ( $x = 84.1 \pm 4.4$ ) than *L. scorialis* ( $x = 76.5 \pm 4.3$ ) (Mann–Whitney  $U = 15.0$ ,  $P < 0.01$ ,  $DF = 21$ ); but *L. buergeri* has fewer ventral scales ( $x = 118.7 \pm 4.7$ ) than *L. scorialis* ( $x = 124.0 \pm 6.0$ ) (Mann–Whitney  $U = 36.0$ ,  $P = 0.05$ ,  $DF = 21$ ).

*Liolaemus scorialis* is syntopic with “*L. kriegel*/*Liolaemus* sp. A”, but in addition to the size difference, the latter has more midbody scales ( $x = 94.3 \pm 4.8$ ,  $n = 8$ ) than it (Mann–Whitney  $U = 1.5$ ,  $P < 0.01$ ,  $DF = 16$ ). Moreover, the dorsal scale count range of *L. scorialis* does not overlap with the range of “*L. kriegel*/*Liolaemus* sp. A” (Table 3).

**Table 3.** Scelation and morphological characteristics for the species of the *Liolaemus elongatus-kriegi* complex occurring near *L. scoriialis* sp. n. and *L. zabalalai* sp. n. distribution. Juvenile specimens examined are excluded. Source of data for not examined species are: *L. antumalguen* (Avila et al. 2010a), *L. burmeisteri* (Avila et al. 2012), *L. choique* (Abdala et al. 2010), *L. punnabuidai* (Avila et al. 2004) and *L. tregenzai* (Pincheira-Donoso and Sclaro 2007). (\*) Medina et al. (2013). M = males; F = females.

	<i>L. antumalguen</i>	<i>L. buergeri</i> (M = 5, F = 9)	<i>L. burmeisteri</i>	<i>L. carlosgarini</i> (M = 6, F = 11)	<i>L. choique</i>	<i>L. flavipiceus</i> (M = 5, F = 10)	<i>L. punnabuidai</i>	<i>L. scoriialis</i> sp. n. (M = 7, F = 3)	<i>L. tregenzai</i>	<i>L. zabalalai</i> sp. n. (M = 3, F = 5)
Maximum SVL (mm)	107.8	96.2	85.2	68.8	90.7	95.8	96.0	69.9	90.2	92.0
Midbody scales	72–82	80–100	70–81	80–95	74–88	68–77	67–81	76–90	71–85	90–104
Dorsal scales	70–78	78–91	76–85	68–82	65–81	60–71	70–78	74–81	-	86–96
Ventral scales	105–118	111–125	99–110	112–124	118–135	93–105	-	115–131	-	116–122
Sexual dichromatism	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Slight	Present	Slight
Cloacal region color (males)	Yellowish in some specimens but usually black	Yellowish	Yellowish	Yellowish	Yellowish	Reddish or yellowish in some specimens but usually black	Reddish or yellowish	Yellowish	-	Yellowish
Tail pattern	Absent	Vertebral line with diffuse rings in the tail base	Weak rings	Rings (marked or weak)	Absent	Absent or weak rings	Absent	Rings	Rings	Rings
Preloacal pores on males	3–4	3–4	0–5	0–3	3–4	0	0	3–4	0	3–4 (3–5*)



**Figure 2.** Chilean species of the *elongatus-kriegii* complex that live near the distribution of *Liolaemus scorialis* sp. n. and *L. zabalai* sp. n. **A** *L. buergeri* from El Planchón (type locality, photo by J. Troncoso-Palacios) **B** *L. buergeri* from Altos de Lircay (photo by R. Díaz) **C** *L. carlosgarini* from the road to the Maule Lagoon (type locality, photo by J. Troncoso-Palacios) **D** *L. flavipiceus* from the Maule Lagoon (photo by C. Garín).

There is a black lateral band running from the tip of snout to the groin in “*L. kriegii* *Liolaemus* sp. A”, whereas in *L. scorialis* there is a dark brown lateral band running from the shoulder to the groin.

*Liolaemus scorialis* differs from similar size species of the *elongatus-kriegii* complex as follows. *Liolaemus scorialis* differs from *L. cristiani* because the males of the latter lack preloacal pores and have reddish ventral coloration, whereas males of *L. scorialis* have 3–4 preloacal pores and no reddish ventral coloration.

*Liolaemus scorialis* differs from *L. heliodermis*, because the males of the latter have a black head and sulfur-yellow dorsum (Espinoza et al. 2000), an unique feature in the *Liolaemus* subgenus. Moreover, *L. heliodermis* has 62–69 midbody scales (Espinoza et al. 2000), whereas *L. scorialis* has 76–90.

*Liolaemus scorialis* differs from *L. parvus*, because the latter has 60–77 midbody scales and 96–113 ventral scales (Quinteros et al. 2008), whereas *L. scorialis* has 76–90 midbody scales and 115–131 ventral scales. *Liolaemus scorialis* has a ringed tail, whereas *L. parvus* has weak or absent rings on the tail (Quinteros et al. 2008).

*Liolaemus scorialis* differs from *L. smaug*, because the latter has marked sexual dichromatism with white spots dispersed on the dorsum of males and absent in females (Abdala et al. 2010), whereas both males and females of *L. scorialis* have white spots on the dorsum. *Liolaemus scorialis* has ringed tail, whereas *L. smaug* has weak or no rings on the tail (Abdala et al. 2010). Males of *L. smaug* have bright golden yellow dorsal color, a trait absent in *L. scorialis*.



**Figure 3.** Comparison of the ventral color pattern. **A** *Liolaemus scorialis* sp. n. from type locality, with immaculate gray ventral color **B** *L. carlosgarini* with light gray ventral color and dark inconspicuous spots dispersed.

*Liolaemus scorialis* differs from *L. tulkas*, because the males of the latter have 0–1 preloacal pores (Quinteros et al. 2008), whereas males of *L. scorialis* have 3–4 preloacal pores. Moreover, *L. tulkas* has 63–68 midbody scales (Quinteros et al. 2008), whereas *L. scorialis* has 76–90.

*Liolaemus scorialis* differs from *L. carlosgarini* (Fig. 2), because the males of the latter have 0–3 preloacal pores (present in 50% of the males, these are small and underdeveloped), whereas males of *L. scorialis* have 3–4 well developed preloacal pores. *Liolaemus scorialis* has more ventral scales ( $x = 124 \pm 6.0$ ,  $n = 10$ ) than *L. carlosgarini* ( $x = 115 \pm 4.0$ ,  $n = 17$ ) (Mann–Whitney  $U = 11.0$ ,  $P = 0.01$ ,  $DF = 25$ ). Moreover, *L. scorialis* has brown dorsal color and immaculate gray ventral color, whereas *L. carlosgarini* has light brown-yellowish dorsal color and whitish ventral color with dark inconspicuous spots on the gular region and belly (Figs 2 and 3).

**Description of the holotype.** Adult male. SVL 62.3 mm. Tail length 101.5 mm (not autotomized). Axilla-groin length 26.3 mm. Head length (from the posterior border of the auditory meatus to the tip of the snout) 16.4 mm. Head width (distance between the two ear openings) 11.4 mm. Head height (at the level of ear openings) 6.9 mm. Forelimb length 21.1 mm. Hindlimb length 39.7 mm. Foot length 18.9 mm. Rostral scale wider

(2.5 mm) than high (1.0 mm). Two postrostrals. Four internasals. Hexagonal interparietal scale, with a central, small, and whitish spot marking the position of the parietal eye. Interparietal smaller than parietals, surrounded by six scales; nine scales between the interparietal and rostral (both excluded); 15 scales between occiput and rostral; orbital semicircle complete on the right side, formed by 13 scales, incomplete on the left side; 6–5 supraoculars (left–right); six superciliary scales. Frontal area is divided into six scales (two posterior, one in the center and three anterior); 2 scales between nasal and canthal; preocular separated from the lorilabials by one loreal scale; nasal in contact with the rostral, surrounded by seven scales. There is one row of lorilabials between the supralabials and the subocular. Seven supralabials, the fifth is curved upward without contacting the subocular. Four infralabial scales. Mental scale pentagonal, in contact with four scales; four pairs of postmental shields, the second is separated by two scales. Temporal scales are subimbricated and slightly keeled. There are ten temporal scales between the level of superciliary scales and the rictal level. Three projected scales on the anterior edge of the ear, which are small and do not cover the auditory meatus; auricular scale is wide and is restricted to the upper third of the meatus. Forty gulars between the auditory meatuses. Well developed “Y” shaped lateral neck fold and dorsolateral fold slightly developed. Antehumeral fold present. Midbody scales 88. Dorsal scales of the vertebral zone lanceolate, imbricate, keeled and without mucrons. Dorsal scales of the paravertebral fields more rounded, subimbricate, with more poorly developed keel, without mucrons and with interstitial granules between them. Dorsal scales of the vertebral zone are larger than the ventral scales. Dorsal scales of the paravertebral fields are similar in size to the ventral scales. Dorsal scales 81. Ventral scales are rhomboidal to rounded, smooth, imbricate, and without interstitial granules. Ventral scales 131. There are four preloacal pores. The suprafemoral scales are rhomboidal to rounded, imbricate, and smooth or slightly keeled. Infracemoral scales are rounded, smooth, and imbricate. Supra-antebrachials scales are rhomboidal to rounded, imbricate, and slightly keeled or smooth. Infra-antebrachials are rounded to rhomboidal, subimbricate with few interstitial granules, and smooth. The dorsal scales of the tail are rhomboidal, imbricate, keeled and some with mucrons. The ventral scales of the tail vary from rhomboidal to triangular, and are imbricate and smooth. Lamellae of the fingers: I: 10, II: 17, III: 21, IV: 23 and V: 13. Lamellae of the toes: I: 13, II: 18, III: 22, VI: 29 and V: 20.

**Color of the holotype in life.** Light brown head, with dark brown lines: a “Ω” shaped line between nasal scales and supraocular area, two short stripes on the posterior supraocular areas, an incomplete “O” shaped dark brown line surrounding the interparietal scale, six dark brown short lines on the occipital area. The temporal area is brown with two dark brown horizontal stripes; the ocular area and the cheeks are light gray. Subocular area is gray with two dark brown vertical lines on the middle and posterior edge. Background color of the dorsum is brown. A wide occipital band on the dorsum, formed by twelve transverse dark brown bars; some white scales on the posterior border of these bars. Dark brown lateral band with few yellowish scales dispersed into it, running from the shoulder to the groin; some white scales between the occipital and lateral bands; below the lateral band the flanks are yellowish. Limbs

are brown with dark brown spots and some white scales dispersed. Tail is brown with some white scales dispersed and dark brown rings. Posterior third of the tail is immaculate brown. Ventrally, the throat, belly, limbs and tail are immaculate gray. Rear portion of belly and thighs are yellowish. Preloacal pores orange.

**Variation.** There is no sexual dimorphism in size. In seven males: SVL: 57.4–69.9 mm. Axilla-groin distance: 21.4–28.7 mm. Head length: 15.1–17.2 mm. Head width: 11.2–13.0 mm. Head height: 6.4–8.9 mm. Foot length: 19.7–21.1 mm. Leg length: 37.1–46.2 mm. Arm length: 20.3–26.0 mm. Tail length: 101.6–111.3 mm ( $n = 2$ ; autotomized in the rest). In three females: SVL: 57.3–65.6 mm. Axilla-groin distance: 25.6–32.8 mm. Head length: 15.3–15.8 mm. Head width: 11.1–12.1 mm. Head height: 6.2–6.7 mm. Foot length: 18.7–20.0 mm. Leg length: 37.2–39.0 mm. Arm length: 21.8–22.3 mm. Tail length 88.8–103.1 mm ( $n = 2$ ; autotomized in the rest).

The variation of the scalation in *Liolaemus scorialis* is as follows. Midbody scales: 76–90 ( $x = 82.0 \pm 4.7$ ). Dorsal scales: 74–81 ( $x = 76.5 \pm 4.3$ ). Ventral scales 115–131 ( $x = 124.0 \pm 6.0$ ). Fourth finger lamellae: 21–24 ( $x = 22.7 \pm 1.1$ ). Fourth toe lamellae: 28–31 ( $x = 29.2 \pm 1.4$ ). Supralabial scales: 6–7 ( $x = 6.2 \pm 0.4$ ). Infralabial scales: 4–5 ( $x = 4.7 \pm 0.5$ ). Preloacal pores in males: 3–4. Interparietal scale pentagonal or hexagonal, bordered by 5–9 scales ( $x = 6.7 \pm 1.2$ ).

There is a slight sexual dichromatism, females have no yellowish color on the rear portion of belly and thighs. Males have the same color and pattern described for the holotype with variations only in shade. Females have the same color and pattern described for the holotype, but the background color of the dorsum can be brown or gray. One female lacks a wide occipital band because the transverse dark brown bars are not fused and it has an inconspicuous vertebral stripe. Also, in this female there are no lateral bands, since it has unfused vertical bars on the flanks. The tail has dark brown rings in both sexes. Males have orange preloacal pores. The coloration and pattern of the juveniles are unknown.

**Distribution and natural history.** The northern known distribution limit of the new species is the type locality, near the Laja Lagoon, 1450 m, Biobío Region, Chile (37°21'S – 71°23'W; Fig. 4). At the type locality, this new species was found inhabiting areas composed of sandy ground and volcanic sediments, where large accumulations of different sized igneous rocks protrude from the soil (Fig. 5). These sites correspond to a slag heap of solidified lava. The vegetational cover is low, consisting mainly of high-Andean forbs with species such as *Echium vulgare* and *Verbascum thapsus*, as well as the bush *Ephedra chilensis*. It is an abundant lizard of saxicolous habits. It was observed to be active between 9h00 and 18h00, taking refuge under the volcanic rocks. Also, we observed specimens in several places near the slopes of Antuco Volcano (37°23'S – 71°23'W, 1320 m; 37°23'S – 71°23'W, 1270 m; 37°23'S – 71°25'W, 1074 m) in similar environments. Near the Laja Lagoon, at its upper altitudinal limit (1450 m), this species was found in syntopy with *Phymaturus vociferator* Pincheira-Donoso, 2004. At 1320 m, it was found in syntopy with "*L. kriegii* *Liolaemus* sp. A" and *Diplolaemus sexcinctus* Cei et al., 2003. At its lower altitudinal limit (1074 m), it was found in syntopy with *L. lemniscatus* Gravenhorst, 1838 and *L. tenuis* (Duméril & Bibron, 1837).



**Figure 4.** Distributional map for *Liolaemus scorialis* sp. n., *L. zabalai* sp. n. and the species of the *elongatus-kriegi* complex that inhabit in proximity of its. Asterisk: *L. scorialis* (red = near Laja Lagoon, type locality; orange = La Mula Lagoon). Star: *L. zabalai* sp. n. (light green = road to Los Barros, type locality; blue = distribution in Argentina). Purple circle: *L. carlosgarini*. Yellow triangle: *L. flavipiceus*. Green pentagon: *L. buergeri*. Gray octagon: *L. choique*. Brown hexagon: *L. antumalguen*. Black cross: *L. punmahuida*. Light pink diamond: *L. burmeisteri*. Pink square: *L. tregenzai*.



**Figure 5.** View of the type locality of *Liolaemus scorialis* sp. n., composed mainly of scoria volcanic rock.

Its southern limit of distribution is in La Mula Lagoon (La Araucanía Region, Chile), 48 km South from the Antuco Volcano (37°53'S – 71°22'W), 1600 m. We have no data for vegetation or environment in La Mula Lagoon. In this location, according to the Herpetological Catalog of the Museo de Historia Natural of Concepción (unpublished), *L. scorialis* occurs in syntopy with *L. pictus* (Duméril & Bibron, 1837). However, this report probably actually refers to *L. septentrionalis* Pincheira-Donoso & Núñez, 2005 (fide Vera-Escalona et al. 2012). The Museo de Historia Natural of Concepción also listed an unidentified species of *Liolaemus* (labeled as *Liolaemus monticola* sp., see discussion) and the snake *Tachymenis chilensis* Schlegel, 1837, from La Mula Lagoon.

The intestinal and stomach contents were examined; plant and insect remains were found in the intestine, along with a large number of nematodes of an unidentified species. No remains were found in the stomach. At the time of capture (January) two females had three embryos each and one female had several small oocytes.

***Liolaemus zabalai* sp. n.**

<http://zoobank.org/063D3CC3-0606-4CC4-8216-8F6B2B38CC3C>

Fig. 6

*Liolaemus kriegi*, Donoso-Barros, 1974. Bol. Soc. Biol. Concepción, 47: 287.

*Liolaemus kriegi* (in part), Cei, 1986. Mus. Reg. Scien. Nat. Torino, 4: 230.

*Liolaemus* sp?, Torres-Pérez, 1997. Not. Biol., 5(4): 146.

*Liolaemus kriegi* (in part), Pincheira-Donoso, 2001. Not. Mens. Mus. Nac. Hist. Nat., Chile, 346: 11.

*Liolaemus* sp. A, Morando et al., 2003. Syst. Biol., 52: 179.

*Liolaemus kriegi* (in part), Pincheira-Donoso & Núñez, 2005. Pub. Oc. Mus. Nac. Hist. Nat., Chile, 59: 289.

*Liolaemus kriegi* (in part), Mella, 2005. Guía Camp. Rep. Chil. Zon. Cent., p. 64.

*Liolaemus* sp. A, Medina et al. Cuad, 2013. Herp. 27(1): 27.

*Liolaemus* sp. A, Medina et al., 2014. Biol. J. Linnean Soc. 113: 256.

**Holotype.** SSUC Re 602 (Fig. 6). Near Los Barros, Laja Lagoon, Biobío Region, Chile. (37°31'S – 71°15'W, 1460 m). Collected by J. Troncoso-Palacios, F. Urra and H. Díaz. 07/01/2014.

**Paratypes.** SSUC Re 598. Adult male. SSUC Re 597, 599, 600–01. Four adult females. The same data as the holotype (Figs 6 and 8). MZUC 35607, 39567. One male and one female. Malleco, Antuco Volcano, Los Barros. Unknown coll.

**Etymology.** This species is named after Patricio Zabala, collection manager of the “Colección de Flora y Fauna Patricio Sánchez Reyes, Pontificia Universidad Católica de Chile” (SSUC). We dedicate this species to him because of his support of herpetological research in Chile, allowing us to review and deposit material in SSUC, and especially for his friendship.

**Diagnosis.** *Liolaemus zabalai* belongs to the *kriegi* clade of the *elongatus-kriegi* complex and is closely related to some undescribed species: *Liolaemus* sp. C and *Liolaemus* sp. D; being more distant from the currently described species *L. buergeri*, *L. kriegi* and *L. tregenzai* (Fig. 7). According to Medina et al. (2014), in regards to the species of the *kriegi* clade *L. zabalai* is sympatric only with *L. tregenzai* at the Copahue Volcano.

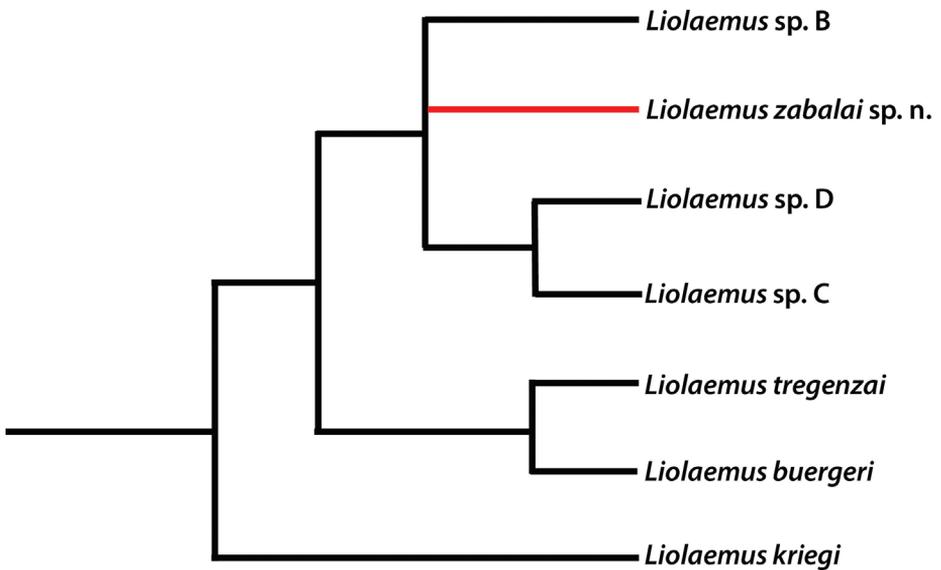
With respect to the species of the *kriegi* clade, *Liolaemus zabalai* differs from *L. tregenzai* because the latter has 71–85 midbody scales and the males have no preloacal pores (Pincheira-Donoso and Scolaro 2007), whereas *L. zabalai* has 90–104 midbody scales and the males have 3–5 preloacal pores. In addition, the green-bluish ventral color of *L. tregenzai* is completely absent in *L. zabalai*. The uncorrected pairwise difference (cyt-b) between the species is 3.09% (Medina et al. 2014).

*Liolaemus zabalai* differs from *L. kriegi* in that the latter reaches 101.1 mm SVL, has reddish cloacal coloration in both sexes and has an unringed tail (Avila et al. 2003), whereas *L. zabalai* is smaller (max. SVL = 92.0 mm), has yellowish cloacal coloration in both sexes and has a ringed tail (in specimens with original tails). The uncorrected pairwise difference between these species is 3.79% (Medina et al. 2014).

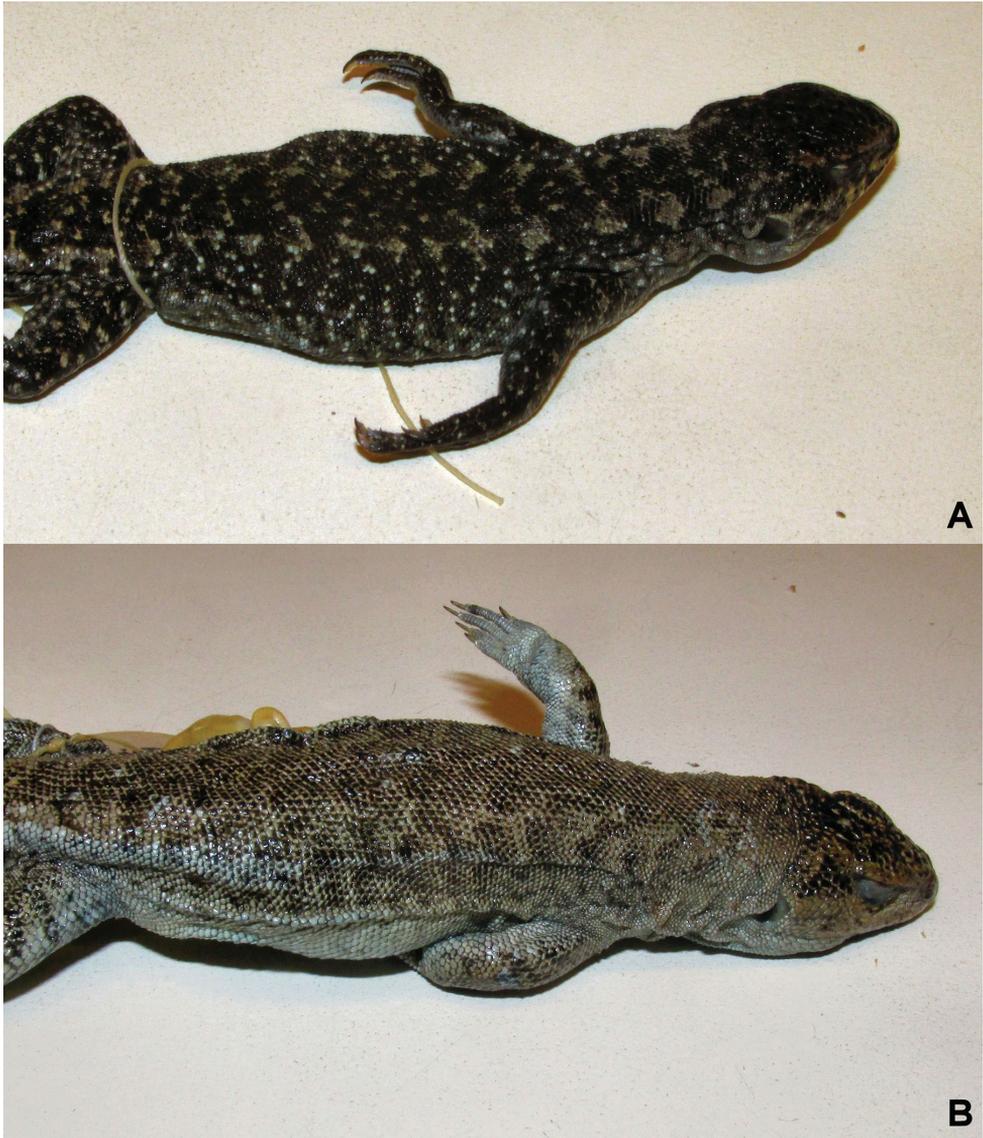
*Liolaemus zabalai* differs from *L. buergeri* in that the latter has fewer dorsal scales (78–91;  $x = 84.1 \pm 4.4$ ,  $n = 14$ ) than *L. zabalai* (86–96;  $x = 89.4 \pm 3.2$ ,  $n = 8$ ) (Mann–Whitney  $U = 19.5$ ;  $P = 0.01$ ,  $DF = 20$ ). *Liolaemus zabalai* has more loreal scales between the nasal and the subocular (4–6;  $x = 4.3 \pm 0.6$ ,  $n = 8$ ) than *L. buergeri* (3–4;  $x = 3.3 \pm 0.5$ ,  $n = 14$ ) (Mann–Whitney  $U = 11.0$ ;  $P < 0.01$ ,  $DF = 20$ ). Also, *L. buergeri* has a vertebral stripe on the tail, whereas *L. zabalai* has a ringed original tail. The limbs in *L. zabalai* are black with dispersed light brown spots, whereas *L. buergeri* has brown limbs with dispersed black spots (Fig. 8). *Liolaemus zabalai* and *L. buergeri* share basically the same dorsal coloration pattern, but this is noticeably more marked and darker in *L. zabalai* (Fig. 8, see discussion). Based on the cyt-b locus, the uncorrected average pairwise difference between *L. zabalai* and *L. buergeri* is 2.94% (Medina et al. 2014), greater than the values reported for other *Liolaemus* widely accepted as valid species



**Figure 6.** *Liolaemus zabalai* sp. n. **A, B** Holotype, male **C, D** Paratype, female **E** Paratype, male **F** Paratype, female. All from the type locality, near Los Barros, Laja Lagoon, Biobío Region, Chile.



**Figure 7.** Phylogenetic position of *Liolaemus zabalai* sp. n. in the *kriegi* clade, based on cytochrome-b (cyt-b) locus according to Medina et al. (2014).



**Figure 8.** Comparison of the dorsal color pattern. **A** *Liolaemus zabalai* sp. n. with marked color pattern and **B** *L. buergeri*, diffuse color pattern.

(see discussion). Also, *L. zabalai* can vocalize, a feature only documented for *L. chilienensis* in the entire genus *Liolaemus* (Labra et al. 2013). Finally, although the ranges overlap, males of *L. buergeri* have 3–4 ( $x = 3.3$ ) precloacal pores, whereas males of *L. zabalai* have 3–5 ( $x = 3.9$ ) precloacal pores (Medina et al. 2014).

Compared to the other species of the *elongatus-kriegi* complex that occur near the known distribution of *Liolaemus zabalai*, the new species may be diagnosed as follows. Males of *L. zabalai* have precloacal pores, whereas males of *L. flavipiceus* and *L. pun-*

*mahuída* lack them (Table 3). *L. zabalai* is larger than *L. scorialis*; and *L. zabalai* has more midbody scales than *L. antumalguen*, *L. burmeisteri* and *L. choique* (Table 3).

**Description of the holotype.** Adult male. SVL: 90.3 mm. Tail length: 92.3 mm (autotomized). Axilla-groin length 39.7 mm. Head length (from the posterior border of the auditory meatus to the tip of the snout) 22.2 mm. Head width (distance between the two ear openings) 16.5 mm. Head height (at the level of ear openings) 11.7 mm. Forelimb length 28.5 mm. Hindlimb length 47.1 mm. Foot length 23.4 mm. Rostral scale wider (4.5 mm) than high (2.2 mm). Two postrostrals. Four internasals. Heptagonal interparietal scale, with a central, small, and whitish central spot marking the position of the parietal eye. Interparietal smaller than right parietal, but bigger than left parietal, surrounded by eight scales: nine scales between the interparietal and the rostral; 14 scales between occiput and rostral; orbital semicircle complete on both sides (formed by 13 scales); 5 supraoculars on both sides; seven superciliary scales. Frontal area is divided into six scales (three posterior, one anterior-left, two anterior-right); 2 scales between nasal and canthal; preocular separated from the lorilabials by one loreal scale; nasal in contact with the rostral, surrounded by six scales. There is one row of lorilabials between the supralabials and the subocular. Seven supralabials, the fourth is curved upward without contacting the subocular. Five infralabial scales. The mental scale is pentagonal and is in contact with four scales. Four pairs of postmental shields, the second is separated by two scales. Temporal scales are subimbricated and smooth or slightly keeled. Nine temporal scales between the level of superciliary scales and the rictal level. Two projected scales on the anterior edge of the ear, which are small and do not cover the auditory meatus. There is no differentiated auricular scale. Forty-two gulars between auditory meatus. Well developed “Y” shaped lateral neck fold with antehumeral and posthumeral folds developed. Dorsolateral fold slightly developed. Midbody scales 90. Dorsal scales on the vertebral zone are lanceolate to rounded, subimbricate, keeled and without mucrons. Dorsal scales on the paravertebral fields are more rounded, subimbricate, smooth or with less developed keels, without mucrons and there are interstitial granules between them. Dorsal scales are smaller than the ventral scales. Dorsal scales 86. Ventral scales are rhomboidal, smooth, subimbricate, and with few interstitial granules. Ventral scales 122. There are three preloocal pores. The suprafemoral scales are rhomboidal, imbricate, and smooth or keeled. Infracemoral scales are lanceolate to rhomboidal, smooth, and subimbricate and with few interstitial granules. Supra-antebrachials scales are rhomboidal to rounded, subimbricate, and keeled or smooth. Infra-antebrachials are rounded to rhomboidal, subimbricate, and smooth. The dorsal scales of the tail are lanceolate to rectangular, subimbricate, keeled or smooth and with few interstitial granules. The ventral scales of the tail vary from lanceolate to triangular, and are subimbricate and smooth. Lamellae of the fingers: I: 11, II: 16, III: 20, IV: 22 and V: 15. Lamellae of the toes: I: 12, II: 16, III: 21, VI: 27 and V: 18.

**Color of the holotype in life.** Black head, with some light brown spots on the supraocular and snout areas. The scales located behind the orbital semicircles are light brown; but the interparietal scale, parietal scales and the scales in contact with the

parietal scales are black. Superciliary scales are light brown with black spots. Temporal scales are light brown; cheeks light gray with some black spots. Subocular is gray with a black vertical line on the middle. Background color of the dorsum is light brown. Wide occipital band on the dorsum, formed by twelve transverse black bars. Very few whitish scales dispersed on the dorsum. Black lateral band bearing a few dispersed whitish scales, running from the tip of snout to the groin. Flanks below lateral band are light brown. Limbs black with dispersed light brown spots. Tail light brown with inconspicuous vertebral stripe in the regenerated zone; occipital black band ends in the first fifth of the tail, remainder with some dispersed black spots and a black vertebral stripe. Throat, belly and ventral surfaces of limbs whitish with dispersed inconspicuous dark dots. Rear portion of the belly and the thighs are yellowish. Ventrally, tail is whitish with a dark gray ventral stripe and diffuse dark gray rings from the cloaca to the midpoint of the tail. Precloacal pores orange.

**Variation.** In three males: SVL: 72.6–90.3 mm. Axilla-groin distance: 32.7–38.6 mm. Head length: 17.6–22.2 mm. Head width: 14.2–16.5 mm. Head height: 9.2–11.7 mm. Foot length: 21.5–23.0 mm. Leg length: 42.1–47.2 mm. Arm length: 24.6–28.5 mm. Tail length: 102.0 mm in one specimen (autotomized in the rest). In three females: SVL: 71.8–90.2 mm. Axilla-groin distance: 32.9–42.7 mm. Head length: 17.9–19.5 mm. Head width: 13.9–16.6 mm. Head height: 9.4–11.1 mm. Foot length: 20.6–24.2 mm. Leg length: 41.5–48.8 mm. Arm length: 24.8–29.4 mm. Tail length: 105–115 mm (in two specimens without autotomized tails).

The variation of the scalation in *Liolaemus zabalai* is as follows. Midbody scales: 90–104 ( $x = 94.3 \pm 4.8$ ). Dorsal scales: 86–96 ( $x = 89.4 \pm 3.2$ ). Ventral scales 116–122 ( $x = 119.5 \pm 2.1$ ). Fourth finger lamellae: 19–22 ( $x = 20.9 \pm 1.0$ ). Fourth toe lamellae: 26–27 ( $x = 26.8 \pm 0.5$ ). Supralabial scales: 6–7 ( $x = 6.6, \pm 0.5$ ). Infralabial scales: 4–5 ( $x = 4.6 \pm 0.5$ ). Interparietal scale pentagonal, hexagonal or heptagonal, bordered by 5–8 scales ( $x = 7.3 \pm 1.1$ ). Precloacal pores in males: 3–4.

There is slight sexual dichromatism; males are slightly darker than females. In general, all specimens have the pattern and color described for the holotype. One female has rusty-colored scales dispersed on the flanks, paravertebral fields and groin. In all specimens, the ventral surface of the throat, belly and limbs are whitish with dark marked or inconspicuous dots dispersed; there is a fragmented midventral stripe on the belly of two specimens. Males and females have a yellowish coloration in the posterior portion of the belly and the thighs (faint in some females). The tail has black rings, marked or diffuse, with a fragmented vertebral stripe in all specimens with complete original tails. Males have orange precloacal pores. The coloration and pattern of the juveniles are unknown.

**Distribution and natural history.** To our knowledge, in Chile this species is only found in the surroundings of the Laja Lagoon. The type locality is near Los Barros, Laja Lagoon, Biobío Region, Chile (37°31'S – 71°15'W, 1460 m, Fig. 9); but we also saw specimens (not collected) on the road to the Laja Lagoon at two localities (37°23'S – 71°23'W, 1320 m; 37°23'S – 71°22'W, 1390 m). The new species was found inhabiting areas of sandy soil with rocks of small and medium size. The vegetational cover is low, consisting mainly of *Ephedra chilensis*. It is an abundant lizard of saxicolous habits. This species was observed active between 11h00 and 18h00, taking refuge in



**Figure 9.** View of the type locality of *Liolaemus zabalai* sp. n.

cavities under the rocks. Near Los Barros, at its upper altitudinal limit (1460 m), this species was found in syntopy with *Diplolaemus sexcinctus*. At the lower altitudinal limit (1320 m), it was found in syntopy with *Liolaemus scorialis*, *Phymaturus vociferator* and *D. sexcinctus*. Two specimens of *L. zabalai* vocalized (squealed) in several occasions in response to the manipulation.

*Liolaemus zabalai* is also found in Argentina (where it has been called “*Liolaemus* sp. A”) at several localities in Neuquén Province (Morando et al. 2003, Medina et al. 2013, 2014).

An analysis of the intestinal contents performed on one specimen, showed that this species is omnivorous, but feeds mainly on plants. At the time of capture (January) the females had no embryos, but three had several small oocytes.

## Discussion

In this work, the taxonomic status of two Chilean populations of the *Liolaemus elongatus-kriegi* complex from the Laja Lagoon have been clarified, here newly described as *L. zabalai* (previously confused with *L. kriegi* and also designed as *Liolaemus* sp. A) and *L. scorialis*. Pincheira-Donoso (2001) recorded two species of the *L. elongatus-kriegi* complex from the same location: *L. kriegi* and *L. buergeri*. Even though we did not examine the three specimens of “*L. buergeri*” listed by Pincheira-Donoso (2001), we believe that these correspond to *L. scorialis*, since the aspect of this new species resem-

bles *L. buergeri* (although it is notably smaller than it) and we did not find additional species of the *elongatus-kriegi* in the vicinity of Laja Lagoon. Also, Troncoso-Palacios et al. (2012) published several photographs of specimens from a population of “*L. buergeri*” from Los Humos, Libertador Bernardo O’Higgins Region, Chile, but unfortunately those specimens were not collected. This population is completely isolated from other populations of *L. buergeri* and some specimens exhibit a completely black ventral coloration, a feature absent in other populations of *L. buergeri* (Donoso-Barros 1966, Pincheira-Donoso and Núñez 2005). A more conclusive study in regard to this population should be conducted. Besides, there is diverse evidence supporting the existence of at least three more undescribed species currently assigned to *L. buergeri* in Argentina (Medina et al. 2013, 2014, Morando et al. 2003).

Assigning *Liolaemus scorialis* to any of the groups (Lobo 2005, Lobo et al. 2010b) or clades (Morando et al. 2003, Avila et al. 2012) proposed for such a diverse lineage of Patagonian lizards is a difficult task, especially taking into account that the phylogenetic studies based on morphological and molecular data disagree, and unfortunately we do not have molecular data for *L. scorialis*. However, it is unlikely that *L. scorialis* belongs to the *leopardinus* group-clade, because it completely lacks “leopard-like” dorsal spots, a distinctive feature of these lizards (Lobo 2005). Also, it is unlikely that *L. scorialis* belongs to the *capillitas* group, because species of this group share two synapomorphies absent in *L. scorialis*: spots in the shoulder region and a red coloration in the cloacal zone (Abdala et al. 2010, Lobo 2005). The *petrophilus* clade (Avila et al. 2012, Morando et al. 2003) includes all species of the *capillitas* group (with the exception of *L. heliodermis*, not sampled) plus *L. austromendocinus*, *L. gununakuna*, *L. parvus* and *L. petrophilus*. However, with the exception of *L. petrophilus* and *L. gununakuna*, all species of the *petrophilus* clade have fewer than 82 midbody scales (Abdala et al. 2010, Avila et al. 2004, Espinoza and Lobo 2003, Quinteros et al. 2008), whereas *L. scorialis* has 76–90 midbody scales. In regards to the *punmahuida* clade (Avila et al. 2010a), included into the *elongatus* group by Lobo et al. (2010b), both species of this clade (*L. flavipiceus* and *L. punmahuida*) have red coloration in the cloacal zone and males lack precloacal pores (Avila et al. 2003, Cei and Videla 2003), features absent in *L. scorialis*. *Liolaemus scorialis* is probably related to the *elongatus* or *kriegi* clades, as some species of these clades occur in the vicinity or in the type locality of *L. scorialis* and have similar counts of midbody, dorsal and ventral scales. Also, some of these species have white dorsal dots, rings on the tail and yellow in the cloacal zone (Abdala et al. 2010, Avila et al. 2010a, 2012, Cei 1986) like *L. scorialis*. A molecular phylogeny including *L. scorialis* is required to clarify this.

In the case of *Liolaemus zabalai* of the *kriegi* clade, the uncorrected pairwise differences between it and other species of the *kriegi* clade are 2.94–3.79%, almost at the limit of the value (3%) proposed for identify candidate species in *Liolaemus* (Breitman et al. 2012). In comparison, other *Liolaemus* lizards widely accepted as valid species show a lower level of differentiation for the mitochondrial gene *cyt-b*, for example: *L. martorii* Abdala, 2003 vs. *L. morenoi* Etheridge & Christie, 2003, 2.73% (Avila et al. 2010b); *L. riojanus* Cei, 1979 vs. *L. multimaculatus* (Duméril & Bibron, 1837), 1.23% (Avila et al. 2009); *L. chacabucoense* Núñez & Scolaro, 2009 vs. *L. kingii* (Bell, 1843), 2.22% (Breitman 2013). *Liolaemus zabalai* can vocalize, a trait only documented for *L. chiliensis*

(Labra et al. 2013) and also taken as diagnostic feature in *Liolaemus* (Pincheira-Donoso and Núñez 2005: 232) and the closely related genus *Phymaturus* (Lobo et al. 2010a: 118). Regarding the morphological diagnosis included in previous studies, Pincheira-Donoso and Núñez (2005) reviewed two specimens of *L. kriegi* from Laja Lagoon (here described as *L. zabalai*), which they described and provided the following diagnosis “the species is very similar to *L. buergeri*, differing in that the latter has a lighter color, brown or dark brown; in combination with a smaller number of keeled scales on the dorsum” (Pincheira-Donoso and Núñez 2005: 289, our translation). Here, we find the same color difference, and expand the differences in scalation; although we found no differences in the number of dorsal scales. Medina et al. (2013) recorded a similar maximum SVL (86.3 mm) compared to us (92.0 mm). Also, Medina et al. (2013) based on a discriminant analysis of several continuous and meristic characters, reported that *L. zabalai* (designated as “*Liolaemus* sp. A” in its study) has sexual dimorphism, with a sample of 21 females and 23 males. We were unable to replicate the statistical analysis to confirm this sexual dimorphism because our sample is small (5 females and 3 males). Also, Medina et al. (2013) recorded 3–5 precloacal pores in the males ( $n = 23$ ), whereas we recorded only 3–4 ( $n = 3$ ). Eventhough we found *Liolaemus scorialis* and *L. zabalai* in syntopy, *L. scorialis* was found mainly in a solid lava slag heap (where it was the only species recorded in this environment), whereas *L. zabalai* was found in bushy-rocky environments together with specimens of *L. scorialis* and other lizards. Regarding the population of “*L. kriegi*” from Cordillera de Curicó in Chile, 35°10'S (Donoso-Barros 1966), we have doubts about its real identity, especially considering that according to Medina et al. (2014) *L. kriegi* is distributed south of 38°40'S latitude (coordinates transformed by us).

Torres-Pérez (1997) recorded two *Liolaemus* sp. from Laja Lagoon. He pointed that one of them has 92 midbody scales, brown color and precloacal pores in males. It is difficult to try an identification, but the midbody scale count match with *L. zabalai*. Torres-Pérez (1997) indicated that the other *Liolaemus* sp. has no precloacal pores. It match only with *L. chillanensis* Müller & Hellmich, 1932, recorded in the Laja Laagon (Pincheira-Donoso and Núñez 2005).

In this study, *Liolaemus ceii* is considered a junior synonym of *L. kriegi*. This synonymy was recommended by Morando et al. (2003) because they did not find genetic evidence to differentiate both species. Recently, Medina et al. (2014) performed a wider genetic study and found that these two species form one lineage, called “*Liolaemus kriegi* + *ceii*”. Because individuals from both type localities show some morphological differences, they proposed two hypothesis: (1) *L. ceii* and *L. kriegi* constitute two species, for which different environments prompted relatively rapid and recent morphological divergence with insufficient time for molecular differentiation; and (2) they are conspecific and show clinal morphological variation owing to local adaptations (Medina et al. 2014). However, the published literature regarding *L. ceii* and *L. kriegi* (Cei 1986, Donoso-Barros 1971) does not include enough morphological comparison between them. We believe that for the moment *L. ceii* should be considered as a junior synonym of *L. kriegi*, because published morphological evidence to support *L. ceii* as full species is insufficient and the results of genetic studies (Medina et al. 2014, Morando et al. 2003) do not support to *L. ceii* as full species.

*Liolaemus chillanensis* was included in the *elongatus* clade by Avila et al. (2010a) and Avila et al. (2012) based on mitochondrial DNA data generated by Torres-Pérez et al. (2009), but at least part of the specimens used as vouchers were misidentified (Troncoso-Palacios, unpublished data). Therefore, in this study we do not consider *L. chillanensis* as a member of the *elongatus-kriegi* complex and we excluded it from our comparisons. Also, we examined one male of *Liolaemus monticola* ssp. (MRC 676) syntopic with *L. scorialis* in La Mula Lagoon, and identified it as *L. neuquensis* Müller & Hellmich, 1939, a species described from Copahue Volcano (Müller and Hellmich 1939b), 15 km E from La Mula Lagoon; being the first record of *L. neuquensis* in Chile.

In summary, this work describes two new species of the *elongatus-kriegi* complex lizards from the vicinity of the Laja Lagoon, in southern Chile, one probably confused with *L. buergeri*: *L. scorialis* and the other with a history of mis-identifications as *L. kriegi* or *Liolaemus* sp. A, for which we provide the formal name *L. zabalai*. Nonetheless, there is certainly still much to discover about the diversity of this group of Patagonian lizards.

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## Appendix I

**Specimens examined.** Museum codes are as follow: LCUC (Laboratorio de Citogenética, Facultad de Ciencias, Universidad de Chile), MNHN-CL (Museo Nacional de Historia Natural, Chile), MRC (Museo de Historia Natural de Concepción), MZUC (Museo de Zoología de la Universidad de Concepción) and SSUC (Colección de Flora y Fauna Patricio Sánchez Reyes, Pontificia Universidad Católica de Chile).

*Liolaemus buergeri*. LCUC 2311. El Planchón, 2370 m. M. Lamborot & M.E. Manzur colls. 07/01/1996. SSUC Re 434–37. El Planchón, road to Teno Lagoon. J. Troncoso-Palacios, L. Negrete & R. Barros colls. January, 2012. SSUC Re 171–180. Maule Lagoon. F. Ferri coll. 20/02/2011.

*Liolaemus carlosgarini*. MNHN-CL 4531–67. Road to Maule Lagoon. C. Garín coll. 22/02/2008. SSUC Re 181–189, 349. Road to Maule Lagoon. F. Ferri coll. 20/02/2011.

*Liolaemus cristiani*. SSUC Re 537. El Peine. J. Troncoso-Palacios coll. 28/11/2011.

*Liolaemus flavipiceus*. MNHN-CL 2118, 2120. Maule Lagoon. C. Veloso & S. Silva colls. MNHN-CL 2167, 2170. Maule Lagoon. J.C. Torres-Mura & H. Núñez. MNHN-CL 4399–07. Laguna del Maule, aguas abajo, 2153 m. C. Garín & G. Lobos colls. 03/03/2008. SSUC Re 169–70. Maule Lagoon. F. Ferri coll. 20/02/2011.

*Liolaemus frassinettii*. LCUC 800–01. Cantillana. Unknown coll. 14/04/1983. SSUC Re 80. Altos de Cantillana. F. Torres coll.

*Liolaemus leopardinus*. MNHN-CL 3437–3439. El Colorado. H. Núñez, C. Garín, V. Meriggio, S. Fox & S. Perea colls. 06/01/2001. MNHN-CL 4025, 4027–28. Farellones. C. Veloso coll. 11/01/1988. MNHN-CL 4890–91. El Colorado. D. Esquerré, M. Palma, S. Fox & E. Santoyo colls. February, 2012. SSUC Re 364. Farellones. F. Ferri coll. 12/10/2010. SSUC Re 365. Farellones. F. Ferri coll. 13/02/2011. SSUC Re 366–67. Farellones. F. Ferri, M.L. Carrevedo & J. Troncoso-Palacios colls. 25/01/2012.

*Liolaemus neuquensis*. MRC 676. La Mula Lagoon, Araucanía Region, Chile. Unknown coll.

*Liolaemus ramonensis*. MNHN-CL 4007–08, 4012, 4015–17. Quebrada de Macul. C. Veloso & P. Espejo colls. 06/03/1987.

*Liolaemus scorialis*. SSUC Re SSUC Re 612–17. 7 km NW of the summit of the Antuco Volcano, near the Laja Lagoon, Biobío Region, Chile. J. Troncoso-Palacios, F. Urrea & H. Díaz colls. 08/01/2014. MRC 675, 677, 680, 682. La Mula Lagoon, Ralco National Reserve. Unknown coll. 01/12/2001.

*Liolaemus ubaghsi*. MNHN-CL 3808–16. Chapa Verde. H. Núñez, C. Garín & D. Pincheira-Donoso colls. 22–23/05/2003. MNHN-CL 1601. Chapa Verde. M. Elgueta coll. SSUC Re 491–92. Tranque Barahona, O'Higgins Region, Chile. R. Thomsom & G. Ugalde colls. 15/04/2008.

*Liolaemus valdesianus*. SSUC Re 129. Cajón del Maipo. Unknown coll. SSUC Re 363. Lo Valdés. F. Ferri coll. 10/01/2011. SSUC Re 559. El Yeso. C. Garín coll. 20/02/2013.

*Liolaemus zabalai*. SSUC Re 597–602. Near Los Barros, Laja Lagoon, Biobío Region, Chile. Collected by J. Troncoso-Palacios, F. Urra and H. Díaz. 07/01/2014. MZUC 35607, 39567. Malleco, Volcán Antuco, Los Barros. Unknown coll.



# A taxonomic review of *Aramides cajaneus* (Aves, Gruiformes, Rallidae) with notes on morphological variation in other species of the genus

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## Abstract

The taxonomy of the polytypic and wide-ranging Gray-necked Wood-rail, *Aramides cajaneus* is reviewed, based on external morphology and voice. Throughout its distribution, there is extensive plumage variation, much of it taxonomically uninformative. However, through three informative plumage characters, as well as morphometric and vocal variation, three phylogenetic species were identified within what is today known as *A. cajaneus*, all of which already had available names: *A. albiventris* Lawrence, 1868, from southern Mexico to northeastern Costa Rica, *A. cajaneus* (Statius Müller, 1776) (*sensu stricto*), from southwestern Costa Rica to Argentina, and *A. avicenniae* Stotz, 1992, from a small section of the coast of southeastern Brazil. *A. albiventris* presents extensive plumage variation, but with no geographic structure. The song of *A. cajaneus* and *A. avicenniae* is strikingly and completely different from the song of *A. albiventris*. A previously unnoticed parapatric pattern of distribution of *A. cajaneus* and its congener *A. saracurá* in southeastern Brazil is described, and we clarify that the name *A. plumbeicollis*, included in the synonymy of *A. albiventris*, was first made available in 1892, rather than in 1888 as is widely referred. In addition, plumage variation in *A. ypecaha*, *A. wolfi*, and *A. mangle* is discussed.

## Keywords

*Aramides wolfi*, *Aramides mangle*, *Aramides ypecaha*, Central America, voice, nomenclature, Wood-rails

## Introduction

The genus *Aramides* (Rallidae), as currently accepted, includes seven species of medium to large rails inhabiting mainly aquatic and semi-aquatic environments throughout most of the Neotropics. They have long bills and legs, mostly gray, black, brown and green plumage, barred underwing coverts and a black tail. Of all the species in the genus the Gray-necked Wood-rail, *Aramides cajaneus* (Statius Müller, 1776), is the most widespread and is found from Mexico to Argentina. It is diagnosable by having an entirely gray neck, which contrasts with its chestnut chest (Ripley 1977, Taylor 1996, Sick 1997, Taylor 1998). However, its plumage is highly variable, especially regarding the colors of the nape, lower chest and back, which led to it currently being recognized as containing nine subspecies, making it the only polytypic species in the genus (Bangs 1907, Hellmayr 1929, Hellmayr and Conover 1942, Ripley 1977, Stotz 1992, Taylor 1996, Taylor 1998).

The taxonomic history of *Aramides cajaneus* is rife with disagreements concerning the allocation of specific or subspecific status to populations, as well as about the morphological characters, diagnoses and geographic limits of these putative taxa. Statius Müller (1776) described *Fulica Cajanea*, based on the bird named “Poule d’Eau de Cayenne” (Cayenne’s water hen), illustrated on plate 352 of Daubenton’s (1765–1781) *Planches Enlumineés d’Histoire Naturelle*. This taxon was included by Pucheran (1845) in his newly described genus *Aramides*, and thereafter became known as *Aramides cajanea*. David and Gosselin (2011) drew attention to the fact that *Aramides* is masculine, whilst “*Cajanea*”, as intended by Statius Müller, is an adjective. Thus the correct agreement is *cajaneus*.

The nine subspecies of *A. cajaneus* can be divided into two groups. The first consists of five subspecies usually considered more closely related to *A. c. albiventris*, and that occur from Costa Rica northwards. It includes *A. c. albiventris*, *plumbeicollis*, *mexicanus*, *pacificus* and *vanrossemi*. The first to be described was *Aramides albiventris*, from Belize and Guatemala, by Lawrence (1868). *A. plumbeicollis* was then described by Zéledon (1892) from northeastern Costa Rica. At the time of their descriptions, both were considered allied to, but separate species from *A. cajaneus*. *A. albiventris* was distinguished from *cajaneus* by its paler chest, black belly and presence of a white band in the lower chest. *plumbeicollis* was distinguished from both *cajaneus* and *albiventris* by its russet mantle. Later, Bangs (1907) considered *plumbeicollis* a subspecies of *albiventris*, and described a new subspecies, *A. albiventris mexicanus*, from Vera Cruz, Mexico. This would be separable from nominal *albiventris* by its overall darker coloration and less distinct white band in the lower chest, but the two subspecies reportedly showed a certain degree of intergradation in Yucatán and Honduras. Miller and Griscom (1921) questioned this intergradation, elevated both *mexicanus* and *plumbeicollis* to full species, and described *A. plumbeicollis pacificus* from Tipitapa, in western Nicaragua, based on its darker overall color and lack of white in the lower chest. The last of the group to be described was *A. vanrossemi* Dickey, 1929, from El Salvador. It

would be distinguished from *albiventris* by its overall paler coloration and green rather than yellow terminal third of the maxilla. Then, for the first time and without presenting any rationale, Peters (1934) and later Hellmayr and Conover (1942) considered all the above-mentioned taxa to be subspecies of *Aramides cajaneus*, a treatment that has been followed by all authors ever since.

The second group of subspecies consists of *A. c. cajaneus* and the three taxa considered more closely related to it, namely *A. c. latens*, *morrissoni* and *avicenniae*. They are distributed from Costa Rica southwards. *A. c. cajaneus* occurs in southern Costa Rica, Panama, and throughout most of South America east of the Andes, except where it is replaced by *A. c. avicenniae* (see below). *A. c. latens* was described by Bangs and Penard in 1918 and *A. c. morrissoni* by Wetmore in 1945. Both are from the Pearl Islands archipelago, off the Pacific coast of Panama, with *latens* found on the islands of San Miguel and Viveros, and *morrissoni* on San José and Pedro González. They would be distinguished from *cajaneus* and from each other by subtle differences in size and overall coloration. The final subspecies, *A. c. avicenniae* was described by Stotz in 1992, from the coast of São Paulo state, southeastern Brazil, based on it having a gray, instead of green, back.

*A. cajaneus cajaneus* has several junior synonyms, erected on the basis of one or very few specimens: *Aramides c. venezuelensis* Cory, 1915, *A. c. peruviana* Cory, 1915, *A. c. salmoni* Chubb, 1918 and *A. c. grahami* Chubb, 1919. None of these, however, was ever accepted as valid after their publication. Another form which has been considered a junior synonym is *Aramides chiricote*, from Paraguay, first described as *Rallus chiricote* by Vieillot (1819) based on Azara's (1805) "*chiricôte*". Unlike the aforementioned names, it did receive consideration in the literature, being recognized as a subspecies by Sharpe (1894), and having its validity discussed, but discarded, by Bangs (1907), Hellmayr (1906, 1929), Hellmayr and Conover (1942) and Stotz (1992). Yet another taxon related to *A. cajaneus* is *A. gutturalis* Sharpe, 1894, based on a single peculiar specimen of uncertain provenance. It was accepted as a full species by Peters (1934) and Hellmayr and Conover (1942), but has since been considered a badly prepared skin of *A. cajaneus* (Meyer de Schauensee 1966, Taylor 1996, 1998).

In contrast to *A. cajaneus*, all other species of *Aramides* are monotypic and have much more restricted distributions. They are also among the least known species of Neotropical rails. Basic descriptive data, such as voice and distribution, are deficient or lacking for some of them (Ripley 1977, Taylor 1998, Vaca et al. 2006, Redies 2010, Karubian et al. 2011). Most significantly, none of them has ever had its morphological variation analyzed.

In light of its complex taxonomic history and the extensive variation in external morphology presented by *A. cajaneus*, its plumage and morphometric variation is reviewed and examine its vocalizations examined in a taxonomic context for the first time. Based on these data, a revised, more adequate taxonomic treatment is proposed for the taxa currently included in it. Plumage variation in some other species of *Aramides* is briefly presented and discussed for the first time.

## Material and methods

800 skins of *Aramides cajaneus* were examined by the authors, including representatives of all its subspecies, deposited in the following institutions: Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil; Museu Nacional da Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil; Museu Paraense Emílio Goeldi (MPEG), Belém, Brazil; Museu de História Natural do Capão da Imbuia (MHNCI), Curitiba, Brazil; American Museum of Natural History (AMNH), New York, USA; Field Museum of Natural History (FMNH), Chicago, USA; Natural History Museum (BMNH), Tring, UK; Muséum National d'Histoire Naturelle (MNHN), Paris, France; and Museum für Naturkunde (ZMB), Berlin, Germany. We examined only through photographs a further 206 specimens, deposited in the following institutions: Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil; Museu de Biologia Prof. Mello Leitão (MBML), Santa Teresa, Brazil; Museo de La Salle (MLS), Bogotá, Colombia; Colección Ornitológica Phelps (COP), Caracas, Venezuela; Carnegie Museum of Natural History (CMNH), Pittsburgh, USA; Museum of Comparative Zoology (MCZ), Cambridge, USA; National Museum of Natural History (USNM), Washington, USA; and University of California Donald R. Dickey Bird and Mammal Collection (UCLA), Los Angeles, USA. Photographs were not taken under standardized lighting conditions, but extensive experience with physical examination of *Aramides* skins (as well as of a wealth of other bird taxa) in many lighting conditions allowed us to confidently discern those photographs that allowed meaningful comparison of plumage from those that did not, and the latter were discarded from the analyses.

A list of all specimens examined, with their locality data, is available online as “Suppl. material 1: Specimens examined”. Among the specimens examined, either in person or through photographs, are the name-bearing type specimens of all the nominal taxa related to *A. cajaneus* mentioned above, except *Aramides chiricote* (Vieillot 1819). The holotype of *Aramides cajaneus* (Statius Müller 1776) is the bird illustrated in Daubenton’s (1765–1781) plate “Poule d’Eau de Cayenne”, and it is not known if it has been preserved as a specimen.

In addition to specimens of *A. cajaneus*, we also examined in person or through photographs 410 skins belonging to all other species of the genus. These were deposited in the same institutions listed above, except for a skin of *A. calopterus* in the Naturhistoriska Riksmuseet (NRM), Stockholm, Sweden and a skin of *A. wolffi* (holotype) in the Muzeum i Instytut Zoologii (MIZ), Warsaw, Poland.

Skins of all species of *Aramides* were qualitatively compared, searching for variation in pattern and color of all plumage regions. To describe colors, color names (capitalized in the text below) and codes from Munsell (1994) were sometimes used. Wing, tail, tarsus and bill height, length and width for *A. cajaneus* skins were all measured, following Baldwin et al. (1931). After delimitating diagnosable units in the *A. cajaneus* complex (see below), morphometric differences between them were assessed

through analysis of variance (ANOVA) or its non-parametric counterpart, Kruskal-Wallis' test. These were followed by the post-hoc multiple comparisons tests Tukey and Dunn's, respectively. The level of significance ( $\alpha$ ) adopted for all tests was 0.05. To evaluate geographical variation in measurements, they were plotted against latitude and longitude. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software 2007) or SPSS 13.0 (SPSS, Inc. 2004). All qualitative and quantitative examinations of skin specimens were conducted by the first author.

92 recordings of *Aramidides cajaneus* vocalizations were also analyzed from within the distribution of five of the nine subspecies. These were mostly songs, recognized by being emitted in duets and being louder and more prolonged than other vocalizations in the species' repertoire. They were obtained from sound archives, namely Macaulay Library, Cornell University, Ithaca, USA (LNS); Fonoteca Neotropical Jacques Vieillard, Universidade Estadual de Campinas, Campinas, Brazil (FNJV); Arquivo Sonoro da Seção de Aves do Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP); and Wiki Aves (WA, [www.wikiaves.com.br](http://www.wikiaves.com.br)); from published compilations (López-Lánus 2009 and Minns et al. 2009), through contribution from collaborators (see acknowledgements); and from the authors' own personal archives. Recordings were analyzed through aural inspection and, for those of good quality, as spectrograms on Raven Pro 1.4 (Bioacoustics Research Program 2011). In each recording, we measured six to nine notes for peak frequency (PF), maximum frequency (MaF), minimum frequency (MiF), bandwidth (BW), and duration (D). These measurements were taken using a frequency resolution of 46.9 Hz and time resolution of 1.06 milliseconds and are presented as mean  $\pm$  standard deviation. All qualitative and quantitative (measurements) analyses of sound recordings were conducted by the first author. A list of all recordings examined is available online as "Suppl. material 2: Recordings examined".

We adopt the General Lineage Species Concept (GLSC; de Queiroz 1998, 2005), which defines species as "lineages of metapopulations evolving separately". This concept acknowledges that speciation is a prolonged process during which the diverging lineages acquire properties (such as diagnosability, reciprocal monophyly, reproductive incompatibility) that can be used in practice for their recognition as distinct species (de Queiroz 1998, 2005). Here, we investigate if such properties can be identified in any subpopulations of what is today understood as *A. cajaneus*. We focus mainly on phenotypic differentiation and diagnosability, and also consider reproductive incompatibility, inasmuch as it can be inferred from differences in song, which plays a major role in avian mating (Catchpole and Slater 1995, Baptista and Kroodsma 2001).

The lists of names in each species account include only the names applicable to each taxon and are thus strictly synonymies, not chresonymies (Dubois 2000). In other words, they do not include variants of spelling or concordance, or different combinations of genus and variations of taxonomic level (specific or subspecific) in the usage of the names. Species diagnoses are given only in relation to the other species in the *A. cajaneus* complex.

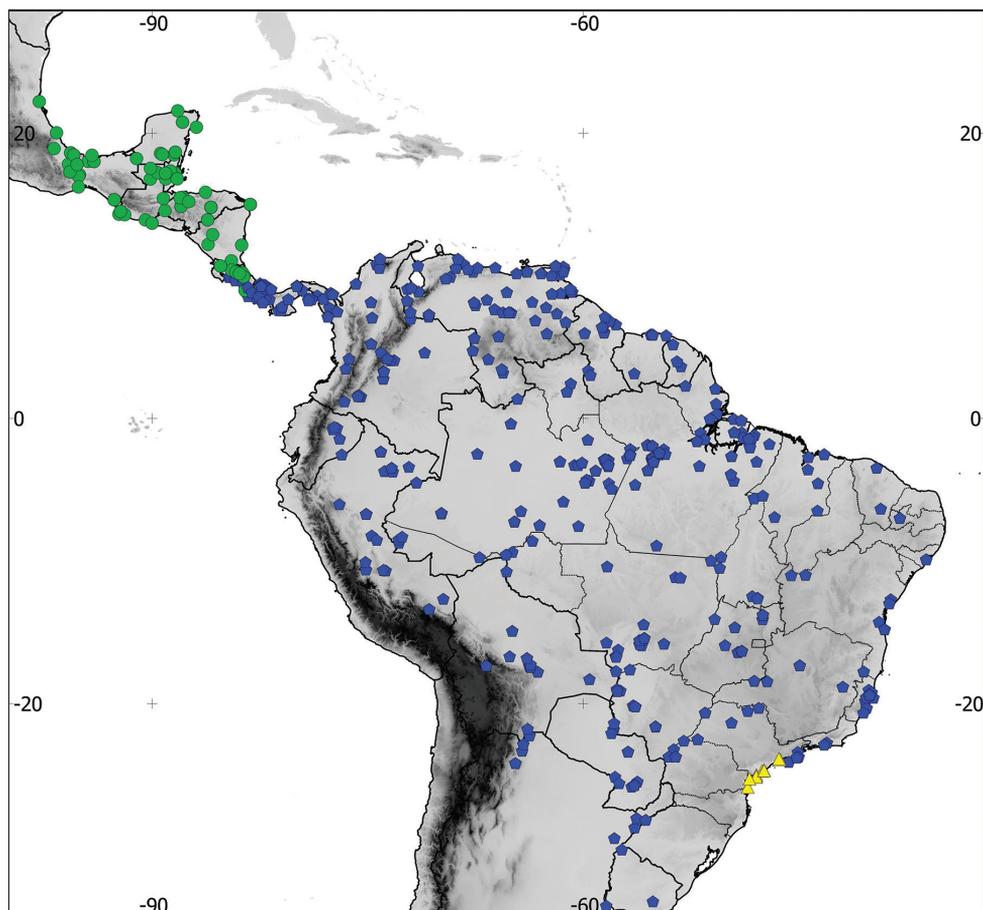
## Results and discussion

*Aramides cajaneus* presents extensive plumage variation throughout its vast range. However, much of this variation is not geographically structured, such that specimens from the same locality are frequently more variable between each other than they are in relation to specimens from a distant locality. These characters are, therefore, not taxonomically informative. An example of this is the chest color, which ranges from Dark Yellowish Brown (10YR 4/6) to Strong Brown (7.5YR 4/6), and varies widely within the same localities, for instance Chapada, Brazil (AMNH 34809 and 58674) and Sarayacu, Peru (AMNH 237512 to 237520). Another example is the amount of greenish or brownish coloration on the rump. For example, in specimens from Lago do Baptista, Brazil, this ranges from totally black (e. g. MZUSP 20923 and 21975) to almost totally brownish green (e. g. MZUSP 21825 and 21803), with several intermediates (e. g. MZUSP 21914 and 22008).

Nevertheless, three plumage characters do vary geographically and allow the delineation of diagnosable clusters of individuals. These are: (1) back color, including the presence and intensity of a brown upper back (mantle); (2) presence of white feathers in the lower chest, separating the chestnut upper chest from the black belly; and (3) presence and intensity of a brown spot in the occiput. Some of the recognized species can also be diagnosed based on remarkable geographical variation in song. Morphometric variation further contributes to characterize them, even though not to their diagnoses, because there is considerable overlap in measurements. Based on these geographically-varying plumage and voice characters, we recognize three species in the *Aramides cajaneus* complex: *A. albiventris*, *A. cajaneus*, and *A. avicenniae* (Figures 1 and 2). In the next sections, we detail the geographical variation in plumage, as well as in vocalizations and morphometry, and discuss the more adequate taxonomy treatments, first by establishing the very well-marked division of the complex into Central American and South American components and then, by delving into variation within each of these components.

### Division of the *Aramides cajaneus* species complex into Central American and South American components

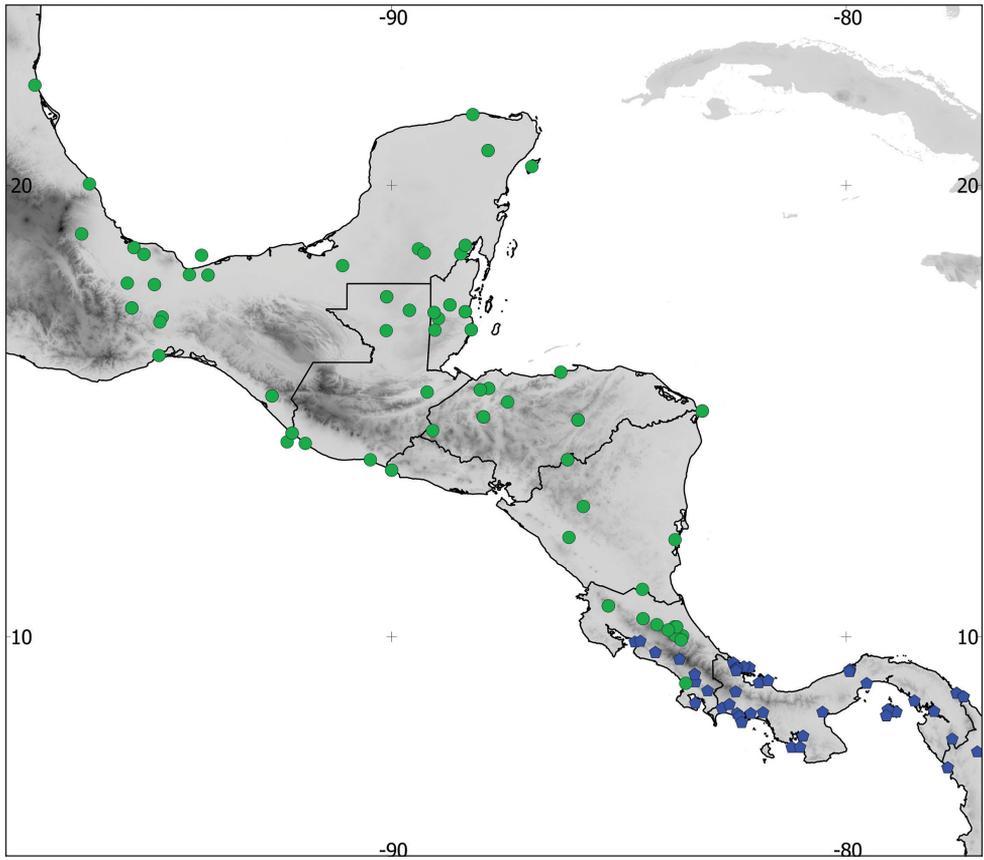
Plumage, vocal and morphometric characters support a clear split between a Central American component (from Mexico south to Costa Rica) and a mainly South American (also including Panama, part of Costa Rica and the Pearl Islands) component in this species complex. In plumage, these components are distinguished from each other, without intermediates, by the much stronger-colored brown nape of Central American birds (Figure 3). Morphometrically, there is an evident discontinuity in variation of bill and tarsus length around 10°N and 83°W, in Costa Rica, where the two components substitute each other (Figure 4). (Other measurements, when plotted against latitude and



**Figure 1.** Distribution of the recognized species in the *Aramides cajaneus* complex, based on examined skins. Green: *A. albiventris* (Central American component), blue: *A. cajaneus* and yellow: *A. avicenniae* (South American component).

longitude, did not show any discernable pattern in variation, and these plots are therefore not shown.) Descriptive statistics for each recognized taxon are presented on Table 1.

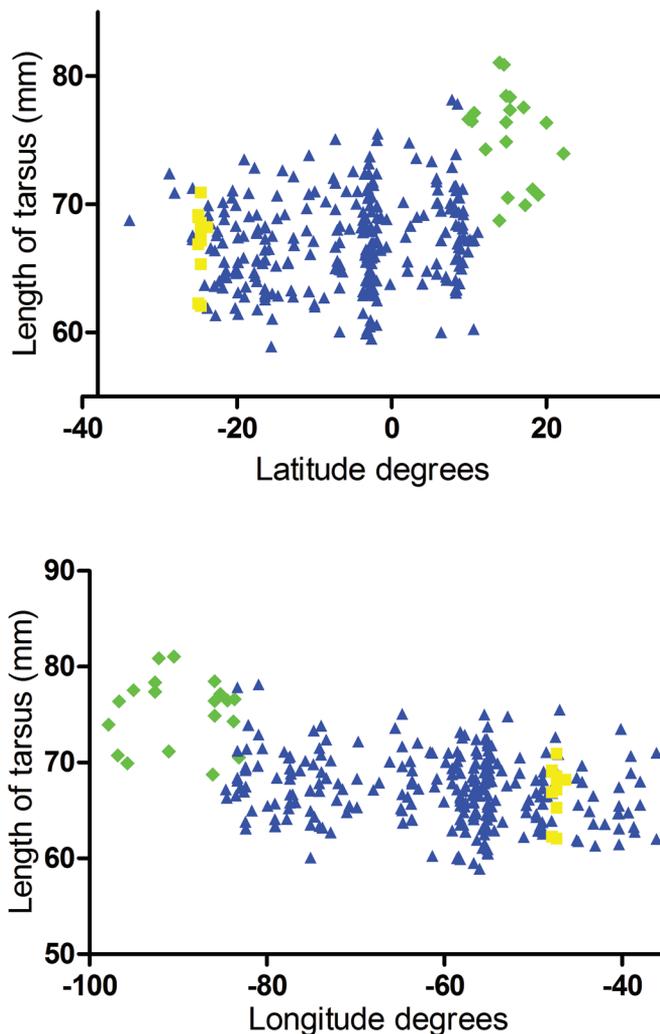
The differences in song are most striking. All available recordings from South America, Panama, and the Caribbean side of Costa Rica (Figure 5), corresponding to the South American component, show a song with a basic phrase consisting of two notes (Figures 6). The first note has ascending-descending-ascending-descending frequency modulation, giving it the approximate shape of an “M” in a spectrogram (PF:  $1603 \pm 66.21$  Hz; MaF:  $1946 \pm 153.5$  Hz; MiF:  $939.2 \pm 96.39$  Hz; BW:  $1007 \pm 178.3$  Hz; D:  $0.154 \pm 0.0288$  sec). The second note is shorter, has a lower frequency, and appears in spectrograms as a simple, slightly ascending line (PF:  $1170 \pm 87.12$  Hz; MaF:  $1372 \pm 112.4$  Hz; MiF:  $924.6 \pm 75.7$  Hz; BW:  $447.8 \pm 83.24$  Hz; D:  $0.1146 \pm 0.0269$  sec). In a typical song bout, performed in a duet, this two-note phrase is repeated in a



**Figure 2.** Detail of the distribution of the recognized species in Central America, based on examined skins. Green: *A. albiventris*, blue: *A. cajaneus*.



**Figure 3.** Nape of South American (the three leftmost specimens) and Central American (the two other specimens) representatives of the *Aramides cajaneus* species complex. Note the much stronger color in the latter.



**Figure 4.** Length of tarsus of specimens in the *Aramides cajaneus* complex plotted against latitude and longitude. Green: *Aramides albiventris*; blue: *A. cajaneus*; yellow: *A. avicenniae*. Note the discontinuity in variation around latitude 10° N and longitude 83° W, in Costa Rica, where the distributions of *A. cajaneus* and *A. albiventris* abut each other.

quick, loud, and lengthy sequence, occasionally interrupted by a short series of lower-pitched notes.

Songs from Belize and southern Mexico (Figure 5), in the range of the second component, are strikingly different (Figure 7). The basic phrase consists of three to four short introductory notes of ascending-descending modulation, followed by three pairs of notes, with similar frequency modulation. In each pair, the first note (PF: 1246±293.92 Hz; MaF: 1436±259.7 Hz; MiF: 878±427.23 Hz; BW: 558±167.5

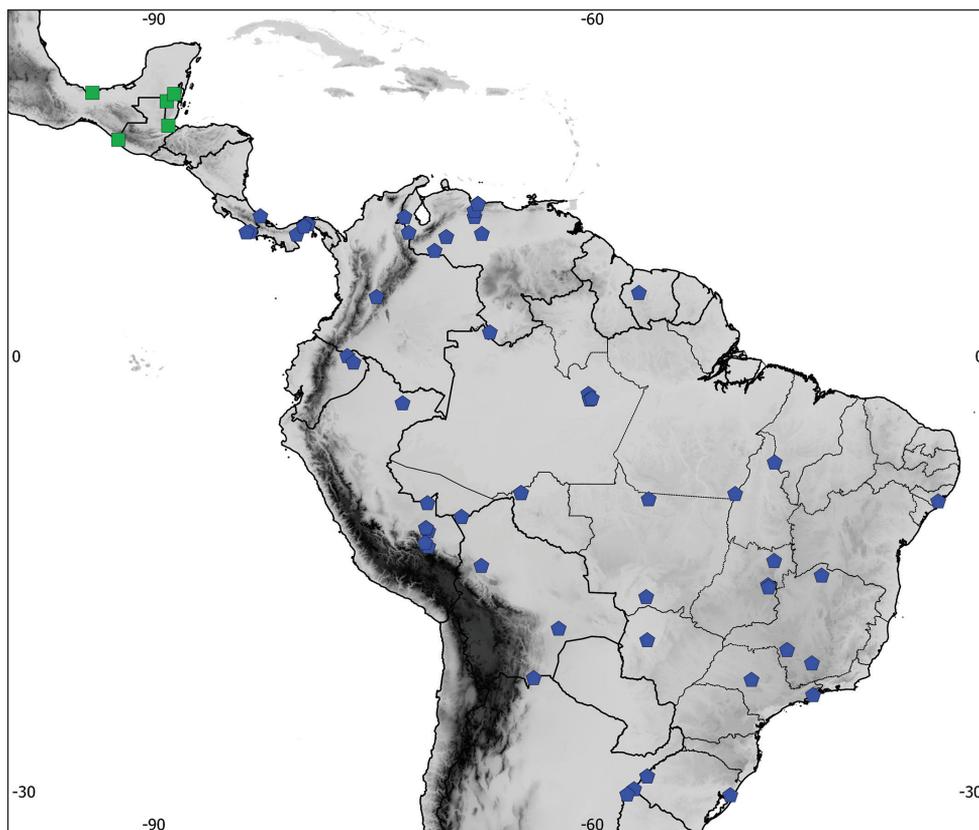
**Table 1.** Mean  $\pm$  standard deviation (first line), range (second line), and sample size (third line) of morphometric variables for each sex of each of the recognized species.

Taxon	Sex	Wing	Tail	Tarsus	Bill height	Bill width	Bill length
<i>A. cajaneus</i>	Males	184.4 $\pm$ 7.98	65.19 $\pm$ 5.73	67.22 $\pm$ 3.51	11.36 $\pm$ 0.74	5.26 $\pm$ 0.43	52.23 $\pm$ 3.19
		159–206	50.51–82.69	58.92–78.16	9.01–13.37	3.70–6.50	38.53–59.90
		277	260	279	231	274	280
	Females	179.0 $\pm$ 7.98	63.02 $\pm$ 6.10	65.07 $\pm$ 4.26	10.86 $\pm$ 0.71	5.05 $\pm$ 0.46	50.19 $\pm$ 2.83
		155–202	49.69–85.79	47.70–76.59	9.02–13.22	3.72–6.94	43.20–59.07
		223	216	224	192	224	223
<i>A. avicenniae</i>	Males	189.4 $\pm$ 7.00	66.22 $\pm$ 3.15	67.07 $\pm$ 2.80	12.56 $\pm$ 0.65	5.73 $\pm$ 0.42	54.74 $\pm$ 1.91
		180–200	59.32–70.80	62.10–70.94	11.6–13.8	5.00–6.20	53.48–56.10
		11	11	11	7	8	10
	Females	182.7 $\pm$ 9.18	66.10 $\pm$ 6.83	65.15 $\pm$ 2.34	11.86 $\pm$ 0.62	5.20 $\pm$ 0.39	51.39 $\pm$ 0.93
		170–195	56.23–75.30	60.20–68.20	11.00–12.62	4.59–5.64	48.70–54.69
		7	9	9	6	7	9
<i>A. albiventris</i>	Males	186.9 $\pm$ 7.59	58.19 $\pm$ 5.39	75.33 $\pm$ 3.54	11.91 $\pm$ 0.84	5.43 $\pm$ 0.58	63.40 $\pm$ 4.23
		173–201	51.04–68.81	68.74–81.06	10.34–12.76	4.26–6.21	54.24–71.06
		18	18	20	11	20	20
	Females	179.05 $\pm$ 8.35	57.80 $\pm$ 6.51	72.81 $\pm$ 4.01	11.16 $\pm$ 0.40	5.22 $\pm$ 0.34	60.54 $\pm$ 4.39
		166–196	48.97–69.07	67.42–80.25	11.54–11.95	4.59–5.88	53.60–68.22
		17	13	19	14	19	17

Hz; D: 0.067 $\pm$ 0.014 sec) is shorter than the second (PF: 2037 $\pm$ 368.2 Hz; MaF: 2294 $\pm$ 358.42 Hz; MiF: 1189 $\pm$ 322 Hz; BW: 1104 $\pm$ 36.42 Hz; D: 0.111 $\pm$ 0.033 sec). Although the sample size is limited, it seems that in a typical session of vocalizations, phrases are delivered in much longer intervals than in the first song type.

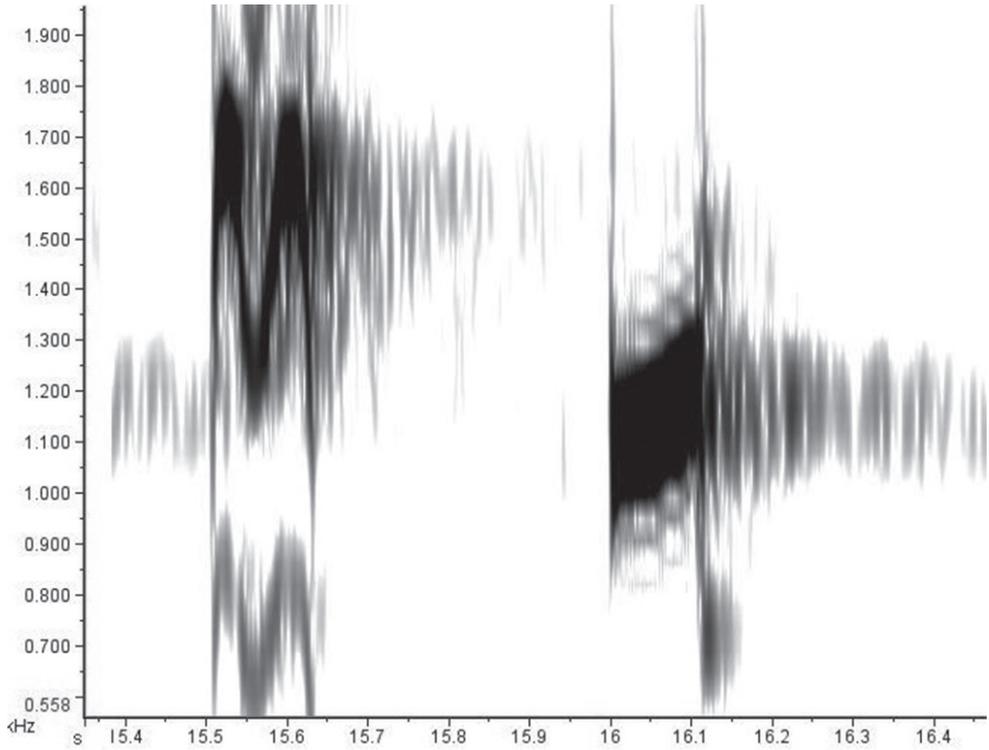
Even though only five recordings of the Central American component were available, the difference between its song and the song of the South American component is striking and consistent. There are neither intermediates nor any elements in each component's vocal repertoires that are even remotely similar to the other's song. In fact, the songs are so distinct that it is impossible even to draw correspondences or hypotheses of homology between their constituent notes. The difference is comparable to that observed between the songs of *A. cajaneus* and other species in the genus, such as *A. saracura* or *A. ypecaba*. Together with the plumage and morphometric differences, this substantiates the recognition of the Central American and South American components as distinct species-level taxa.

The two components are segregated by the Costa Rican mountain ranges, part of the Chorotega Volcanic Front (CVF) that divides lower Central America into Caribbean and Pacific catchments. This is congruent with the identification of the CVF as the location of a major phylogeographic break for several animal taxa in lower Central America (Bagley and Johnson 2014). In addition, the Costa Rican mountains are known to segregate several sister taxa of birds, such as *Amazilia decora* and *Amazilia amabilis* (Trochilidae), *Pteroglossus torquatus* and *Pteroglossus frantzii* (Ramphastidae), *Carpodectes nitidus* and *Carpodectes antoniae* (Cotingidae), among others (Zeledón 1892, Stiles and Skutch 1994).

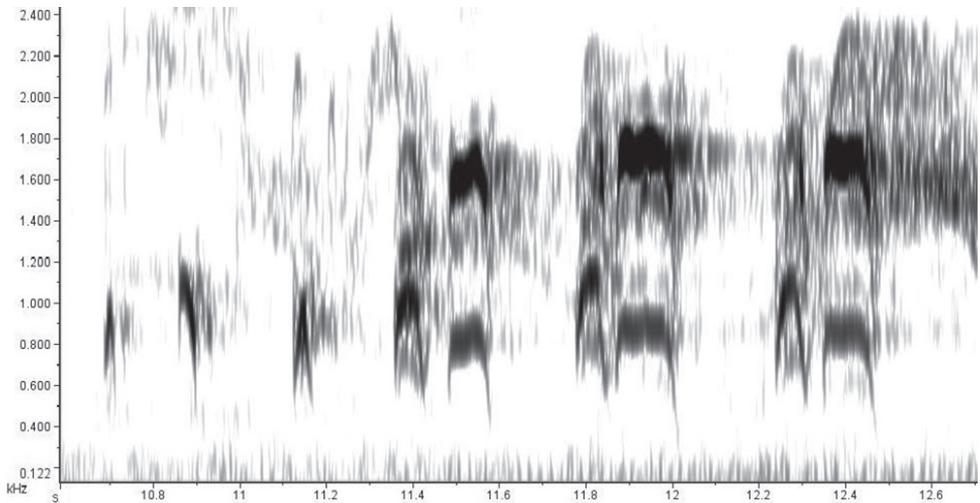


**Figure 5.** Distribution of the analyzed song recordings of the South American (blue), and Central American (green) components of the *Aramides cajaneus* species complex. Their songs are strikingly different; see text for details.

There is one specimen that could potentially falsify the parapatric pattern described above. FMNH 30363 is clearly assignable to the Central American component, having a strong brown nape, but is labeled as coming from El Pozo, Puntarenas province, in the Pacific side of Costa Rica, where only birds belonging to the South American component are supposed to be found. There is reason, however, to believe that this specimen has been mislabeled. It, as well as a typically South America component specimen (FMNH 30364), was collected, according to their labels, by M. A. Carriker in 1907. The label of FMNH 30364 has the precise day and month of collection (June 29), but the label of FMNH 30363 has only the year, which already suggests that there may have been some sort of confusion and loss of information between its collection and its final labeling at the FMNH. Adding to the suspicion that this specimen was not collected in El Pozo is the fact that in 1910 Carriker published an annotated list of the birds of Costa Rica in which he recounts having indeed collected in El Pozo in June 1907. Curiously, however, under *A. albiventris plumbeicollis*, where this specimen could be expected to have been listed, he lists several specimens, but none coming



**Figure 6.** Spectrogram of the typical phrase of the song of the South American component of the *Aramidides cajaneus* species complex (LNS 51765). Note that this spectrogram is not in the same scale as the spectrogram in Figure 7.



**Figure 7.** Spectrogram of the typical phrase of the song of the Central American component of the *Aramidides cajaneus* species complex (LNS 23152). Note that this spectrogram is not in the same scale as the spectrogram in Figure 6.



**Figure 8.** Left: Ventral view of typical specimens of the Central American morphotypes 2 (AMNH 103264) and 1 (AMNH 776255), respectively. Right: dorsal view of same specimens.

from El Pozo. Besides, he writes about this taxon: “Confined entirely to the Caribbean lowlands, and probably only in the northeastern part, since there are no records of its presence in southeastern Costa Rica”. If Carriker had indeed collected a specimen with characters of *A. albiventris plumbeicollis* in the Pacific side of Costa Rica just three years earlier, it is very unlikely that he would fail to list it, and write that the taxon is found only in the Caribbean lowlands. Therefore, the information on the label of FMNH 30363, including locality data, is under suspicion, and this specimen does not falsify the role of the Chorotega Volcanic Front in segregating the *A. cajaneus* species complex into two components.

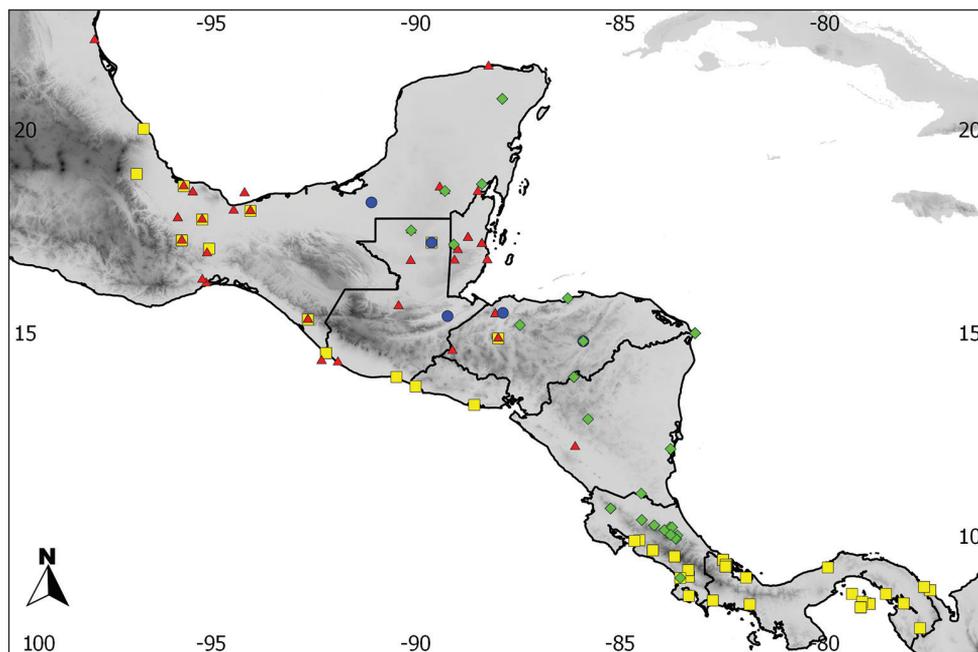
### Variation and taxonomy in the Central American component

Two basic plumage morphotypes can be recognized in the Central American constituent of the *Aramides cajaneus* species complex (Figure 8). In Morphotype 1, found from western Honduras northwestwards to the extreme of the complex’s distribution in Mexico, there never is an homogeneous, conspicuous brown mantle, even though some birds do have a dull brown mantle, fainter along the midline, and there always are white

feathers in the lower chest, in variable extension. In contrast, in Morphotype 2, found from eastern Honduras southeast to the Caribbean side of Costa Rica, there always is a homogeneous brown mantle, and there never are any white feathers on the lower chest, even though some birds do have in that area paler feathers than in the mid and upper chest, but not white. The characters of Morphotype 1, as well as comparison with type specimens, reveal that it is referable to *Aramides albiventris* Lawrence (syntypes from Belize and Guatemala). Morphotype 2, on the other hand, agrees with the description and holotype of *Aramides plumbeicollis* Zeledón (type locality: Jimenez, Costa Rica).

However, in spite of the characters noted above, the distinction between the two morphotypes is doubtful and their recognition as distinct taxa is not warranted, because there are many specimens that blend characters of the two, in various combinations. Some, such as AMNH 393516, from Ocos, Guatemala, have the white chest feathers of Morphotype 1, and the full chestnut mantle of Morphotype 2. Conversely, others, such as AMNH 471954, from Mts. La Cumbre, Honduras, lack both the white lower chest feathers and the chestnut mantle. These intermediate specimens are found mainly in Honduras, Guatemala and Belize, and Quintana Roo, Campeche and Yucatán states in southwestern Mexico but also, in fewer numbers, further northwest (four specimens in Vera Cruz and Oaxaca) and south (two specimens in Costa Rica). In many cases, the intermediate specimens occur in the same localities as either “pure” morphotype, or even the two morphotypes and intermediates all together, such as in El Boquerón, in center-eastern Honduras. No particular geographic pattern of plumage variation is noticeable throughout the extensive area of intergradation (Figures 9 and 10).

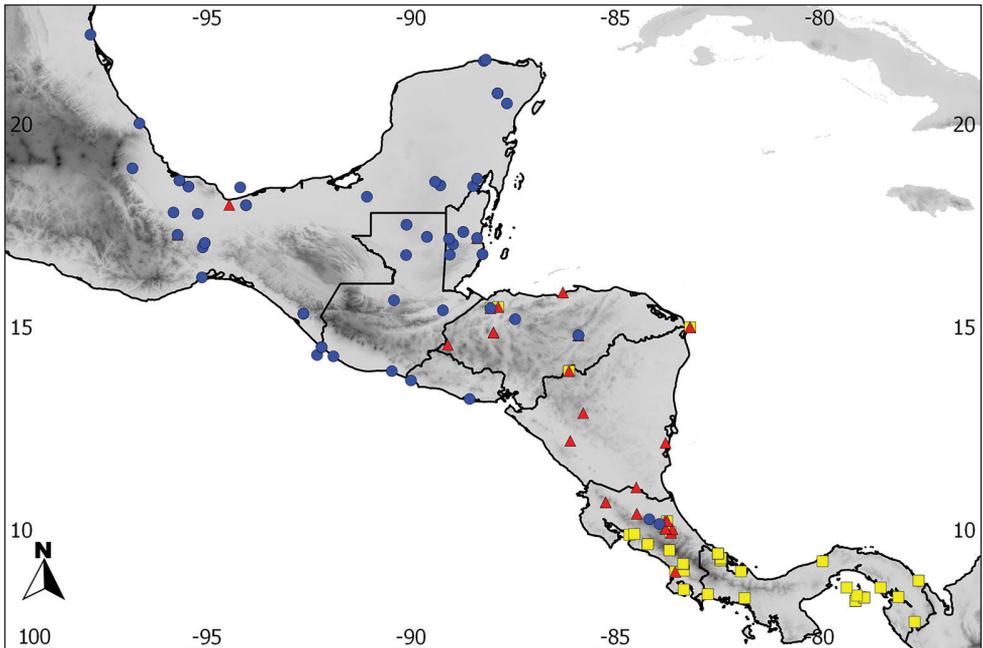
Occurrence of intermediates or hybrids, by itself, does not preclude recognition of two populations as separate species, as long as the variation is not clinal and specimens from outside the intergradation zone maintain their diagnosability (Helbig et al. 2002). In the present case, plumage variation does not appear to be clinal (even though tarsus and culmen measurements, when plotted against latitude and longitude, do hint at clinal variation, the length of both increasing towards north and west; Figure 4). However, the zone of intergradation is too extensive, and intergrades too numerous, to allow recognition of two evolutionarily units. Also due to these intermediates, diagnosability between Morphotypes 1 and 2 is not absolute anywhere in Central America. Unfortunately, no songs from within the range of Morphotype 2 were available, so vocal data cannot be used to inform a decision on the taxonomic status of these populations. Therefore, based on the data we currently have at hand, it appears that the two morphotypes are deeply connected, and cannot be considered distinct evolutionary nor taxonomic units. Thus, we propose that the Central American component of the *A. cajaneus* species complex be recognized as a single species, *Aramides albiventris* Lawrence, 1868, with *Aramides plumbeicollis* Zeledón, 1892 (see ahead for a discussion on the date of its publication) as a junior synonym. At the same time, we also emphasize the importance of further study of these populations, in order to better understand the genetic and historical processes underlying this very complex scenario of phenotypic variation.



**Figure 9.** Mapping of the variation in the mantle of individuals of the *Aramides cajaneus* complex in Central America. Yellow: the upper back has no distinct coloration in relation to the mid and lower back. Red: a faint brownish coloration is present in the sides of the upper back. Blue: a faint brownish tinge is present across the upper back. Green: a complete, conspicuous brownish-orange mantle is present. Notice the lack of any discernable pattern in variation (see text for details).

Regarding the other subspecies currently recognized in Central America, Miller and Griscom (1921) described *A. plumbeicollis pacificus*, based on a single specimen (AMNH 143684) from western Nicaragua. This specimen presents a slight indication of a chestnut mantle, but it is notably more tenuous along the midline, and it has no pure white feathers on the lower chest. It is one of the intermediate specimens between the two *A. albiventris* morphotypes, and thus *A. plumbeicollis pacificus* is a synonym of *A. albiventris*.

The characters used by Bangs (1907) and (Dickey 1929) to describe, respectively, *A. albiventris mexicanus* and *A. vanrossemi* do not support the recognition of these taxa when a large series of specimens is examined. Their supposed diagnostic characters in relation to *albiventris* vary widely throughout southern Mexico and Guatemala. For example, one of the putative diagnostic characters of *A. mexicanus* would be a narrower and more fulvous (instead of white) band in the lower chest. However, the extension and exact tone of the pale feathers in the lower chest are variable throughout the distribution of *A. albiventris*. AMNH 393517, from Ocos (Guatemala), for example, presents a wide, pure white band, while AMNH 393518, from the same locality, presents only a few pure white feathers, the rest of the band being yellowish white, and



**Figure 10.** Mapping of the variation in the lower chest of individuals of the *Aramidides cajaneus* complex in Central America. Yellow: no white or paler feathers in the lower chest. Red: paler chestnut, but not white, feathers are present in the lower chest. Blue: white feathers present in the lower chest.

is in this respect very similar to AMNH 471952, from northern Vera Cruz (within the supposed distribution of *A. mexicanus*) and FMNH 110121, from northern Guatemala. Two specimens from Sarabia, Oaxaca (AMNH 776255 and 776256), also within the supposed distribution of *A. mexicanus*, are very different from each other in the amount of white feathers in the lower chest. Therefore, this character is too variable in southern Mexico and adjacent regions to be taxonomically informative. A similar situation is presented by the other putative diagnostic character of *A. mexicanus*, “all the colors darker” (Bangs 1907). In fact, the holotype of *A. mexicanus* (MCZ 102281) does not in any way stand out from the range of individual variation observed in *A. albiventris*, and they are therefore synonyms.

Dickey (1929) described *Aramidides vanrossemi* based on a single specimen (UCLA 18750) from Barra de Santiago, Ahuachapan, El Salvador. This specimen, too, does not depart significantly from the range of individual variation seen throughout the range of *A. albiventris*. Contrary to the stated by Dickey, it is not “slightly paler throughout”. Also, the author’s statement that it had “lake red instead of yellow” irises is unjustified, given that all birds in the *Aramidides cajaneus* complex have red irises, as attested by specimen labels and abundant photographs available online (Internet Bird Collection; <http://ibc.lynxeds.com/>). Similarly, the statement that the “terminal third of the maxilla [is] green instead of yellow” does not make sense as this too is typical of the whole complex. Thus, *A. vanrossemi* is also here considered a junior synonym of *A. albiventris*.



**Figure 11.** Specimens of *A. avicenniae* (the rightmost specimen) and *A. cajaneus* (all others) from Brazil. Note the homogeneous grey coloration in the hindneck and back of *A. avicenniae*, while in *A. cajaneus* the back is always greener than the hindneck.

### Variation and taxonomy in the South American component

Two taxa can be identified in the South American component of the *Aramides cajaneus* species complex: *Aramides cajaneus* (Statius Müller, 1776), *sensu stricto*, found from Costa Rica south to Uruguay and northern Argentina; and *Aramides avicenniae* Stotz, 1992, found in a small part of the coast of southeastern Brazil.

*A. avicenniae* is distinguished from *A. cajaneus* by its gray, instead of green, back and its more greenish-gray upper wing-coverts. Throughout the distribution of *A. cajaneus*, back color is somewhat variable and even tends towards grayish-green in several specimens from the southwestern part of its distribution and from the northern coast of São Paulo state, not far from the range of *A. avicenniae*. Nevertheless, when specimens of *A. avicenniae* and even the grayest-backed specimens of *A. cajaneus* are placed side-by-side, there is a clear discontinuity in the color of their backs (Figure 11). In specimens of *A. cajaneus* from Ilha dos Búzios, Ilha Alcatrazes and Ubatuba, on the northern coast of São Paulo, the hindneck and upper back are clearly of different colors, even if in some of them the back is darker than the average in *A. cajaneus*. On the other hand, the upper back and the neck are display the same tone of gray in specimens from the São Paulo coast south of Santos (*A. avicenniae*). These patterns demonstrate that *A. avicenniae* is not merely the end of a cline, nor a variation of *A. cajaneus*, and it is hereby regarded as a full species.

Both sexes of *A. cajaneus* (*sensu stricto*) have significantly smaller bill height than *A. avicenniae*, and males have significantly smaller bill width. In addition, the two are significantly smaller than *A. albiventris* in tail length, tarsus length and bill length of both sexes. (Tables 1–3).

Bangs and Penard (1918) described *A. c. latens*, from the islands of San Miguel and Viveros, in the Pearl Island archipelago off the Pacific coast of Panama. It was distinguished from *A. cajaneus* by its smaller size and overall paler plumage. *A. c. morrissoni* was described from the islands of San José and Pedro González, in the same archipelago, by Wetmore (1946), as being similar to *latens*, but told apart by its darker back and hindneck. However, all the specimens from the Pearl Islands examined, including the types of both subspecies (MCZ 114297 and USNM 376059, respectively), fall within the variation observed for *A. cajaneus* and these names are thus treated as junior synonyms. These synonyms of *A. cajaneus*, along with *Rallus chiricote*, *A. c. venezuelensis*, *A. c. peruviana*, *A. c. salmoni* and *A. c. grahami*, are probably the result of overemphasis on minor individual plumage variations and lack of adequate and geographically comprehensive sampling.

### **The distribution of *A. cajaneus* in southeastern Brazil**

When the distributions of *A. avicenniae*, *A. cajaneus* and their congener *A. saracura* (Spix, 1825) are mapped together, it is notable they have almost perfectly parapatric distributions, a pattern never before remarked on. Contrary to what is indicated in several reference works (e. g. Ripley 1977, Taylor 1996, Taylor 1998, Erize et al. 2006, Sigrist 2009), *A. cajaneus* is absent from an extensive part of interior southeastern Brazil and from the Argentine province of Misiones. This area corresponds almost exactly to the distribution of *A. saracura* (Figure 12). *Aramides saracura* and *A. cajaneus* (or its substitute *A. avicenniae*) both occur on the coast of this region, but in that case *A. cajaneus* and *A. avicenniae* are mainly found in mangroves, a habitat not occupied by *A. saracura* (Taylor 1998). Even though *A. saracura* is usually considered more of a forest dweller than *A. cajaneus* (Taylor 1998), it is possible that their ecological preferences are not different to the point of allowing sympatry. A hypothesis derived from this distribution pattern is that *A. saracura* might have been the implied in the differentiation between *A. avicenniae* and *A. cajaneus*. Its presence might have acted as an ecological barrier between inland and coastal populations of *A. cajaneus*, leading to a process of peripatric speciation that culminated with the emergence of *A. avicenniae*.

### **A clarification regarding the date of description of *A. plumbeicollis***

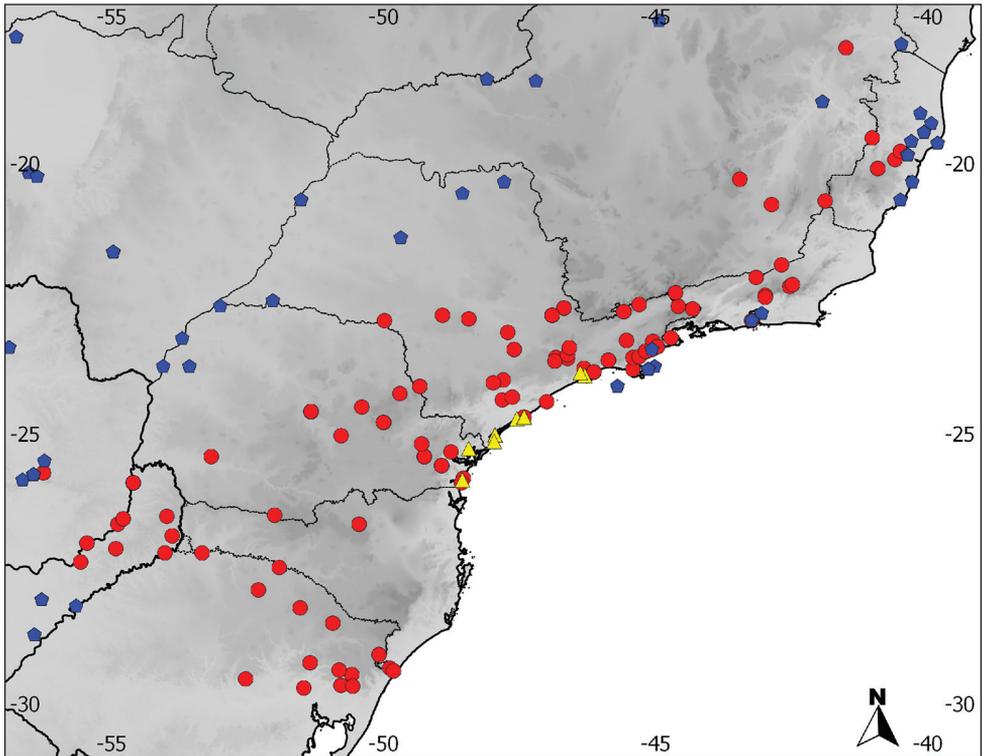
Even though we do not recognize *A. plumbeicollis* as a valid taxon, a clarification is needed regarding this name, given that it is nomenclaturally available and most references have a wrong publication date for it. Hellmayr and Conover (1942) cite the de-

**Table 2.** Results of the ANOVA (parametric) or Kruskal-Wallis (KW; non-parametric) tests comparing the three recognized species. Significant p-values (< 0.05) are in italics.

	Wing		Tail		Tarsus		Bill height		Bill width		Bill length	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Test	KW	KW	ANOVA	KW	ANOVA	KW	KW	KW	ANOVA	KW	KW	KW
p	0.1108	0.6421	<0.0001	0.0019	<0.0001	<0.0001	0.0002	0.0015	0.0036	0.1302	<0.0001	<0.0001

**Table 3.** Results of the post-hoc pairwise comparison tests (Tukey or Dunns) between the recognized taxa. Ns: not significant (p>0.05); \*: 0.05>p>0.01; \*\*: 0.01>p>0.001; \*\*\*: p<0.001.

	Wing		Tail		Tarsus		Bill height		Bill width		Bill length	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
<i>cajaneus</i> × <i>avicenniae</i>	ns	Ns	ns	ns	ns	***	***	**	**	ns	ns	ns
<i>cajaneus</i> × <i>albiventris</i>	ns	Ns	***	**	***	ns	ns	ns	ns	ns	***	***
<i>avicenniae</i> × <i>albiventris</i>	ns	Ns	**	**	**	ns	ns	ns	ns	ns	*	**



**Figure 12.** Distribution of *A. cajaneus* (blue), *A. avicenniae* (yellow) and *A. saracura* (red) in southeastern Brazil. Note the parapatric distribution pattern.

scription of *Aramides plumbeicollis* as “*Anal. Mus. Nac. Costa Rica, 2, p. 3, 1888*”, and a similar citation is given by Ripley (1977). Taylor (1996), Taylor (1998) and Dickinson and Remsen (2013), also have the year as 1888, but without the full reference. However, a careful examination of the relevant publications reveals that the name *Aramides plumbeicollis* was first published, under the rules of the Code, only in 1892.

The name *Aramides plumbeicollis* was first used in a catalogue of the birds of Costa Rica in tome 1 of the *Anales del Museo Nacional—República de Costa Rica* (Zeledón 1888: 131). In this publication the name is not associated with any definition or description of the taxon to which it refers. There is only a footnote that reads: “This species is described on page 3, Tome II of these Annals, Year 1888” (our translation from the Spanish original). According to Article 12 of the Code, names published before 1931 without a description or definition are considered available as long as they are associated with an indication of the animal they refer to. A reference to a past publication fulfills this requirement, but a reference to a future publication does not. Therefore, *Aramides plumbeicollis* Zeledón, 1888, as it appears in this catalogue, is a *nomen nudum*.

Subsequent authors always gave 1888 as the date of the species’ description, probably assuming, based on Zeledón’s (1888) footnote, that *Aramides plumbeicollis* was indeed described in tome 2 of the *Anales del Museo Nacional—República de Costa*

Rica. However, no exemplars of this publication could be found in the library of the Museo Nacional de Costa Rica (Adelina Jara, librarian at the Museo Nacional de Costa Rica, pers. comm.) and this volume was actually never published (Anonymous 1892, Chaves and Bolaños 2011). In fact, following tome 1, the publication was merged with the Anales del Instituto Físico-Geográfico Nacional de Costa Rica, giving rise to a new series titled Anales del Instituto Físico-Geográfico y del Museo Nacional de Costa Rica. However, the numbering of this new series continued with that of the Anales del Instituto Físico-Geográfico Nacional, and its first tome, published in 1892, but referring to 1890, is tome 3 (Anonymous 1892). It is on page 134 of this tome that the description of *A. plumbeicollis* is found, and this is the first time in which that name is made available under the rules of the Code.

## Taxonomic accounts

### *Aramides cajaneus* (Statius Müller, 1776)

*Fulica Cajanea* Statius Müller, 1776. *Natursystems Supplements*, p. 119. Based on “La Grande Poule d’Eau de Cayenne” from Buffon (1781), which is illustrated in the *Planches Enlumineés d’Histoire Naturelle* by L. J. M. Daubenton (plate 352). Type locality: “Caienne” (Cayenne, French Guyana).

*Fulica major* Boddaert, 1783. *Table des Planches Enlumineéz d’Histoire Naturelle de M. D’Aubenton*, p. 21. Based on Buffon’s (1781) “La Grande Poule d’Eau de Cayenne”.

*Fulica cayennensis* Gmelin, 1789. *Systema Naturae*, 13th edition, v. 1, part 2, p. 700. Based on Latham’s (1785) “Cayenne Gallinule” and Buffon’s (1781) “La Grande Poule d’Eau de Cayenne”. Type locality: “Guianae et Cayennae”.

*Fulica ruficollis* Gmelin, 1789. *Systema Naturae*, 13th edition, v. 1, part 2, p. 700. Based on Latham’s (1785) “Black-Bellied Gallinule”. Type locality: “Cayenna”.

*Rallus chiricote* Vieillot, 1819. *Nouveau Dictionnaire d’Histoire Naturelle*, v. 28, p. 551. Based on Azara’s (1805) “Chiricóte”. Type locality: “Paraguay”. Azara’s “Chiricóte aplomado” which Vieillot (1819) considered a variant of his *R. chiricote*, is actually *Aramides saracura*.

*Rallus maximus* Vieillot, 1819. *Nouveau Dictionnaire d’Histoire Naturelle*, v. 28, p. 555. Based on Latham’s (1785) “Cayenne Gallinule”, Gmelin’s (1789) *Fulica cayennensis* and Buffon’s (1781) “La Grande Poule d’Eau de Cayenne”. Type locality: “Cayenne et [...] Guyane”.

*Gallinula ruficeps* Spix, 1825. *Avium Species Novae*, tome 2, p. 74 and plate 96. Type specimen in the Munich museum, not examined. Type locality: “Provincia Rio de Janeiro” (Rio de Janeiro state, Brazil).

*Rallus hydrogallina* Lesson, 1831. *Traité d’Ornithologie*, p. 536. Based on Gmelin’s (1789) *Fulica cayennensis* and Buffon’s (1781) “La Grande Poule d’Eau de Cayenne”. Type locality: “Cayenne” and “Brésil”. The supposed juvenile with slate underparts (“dessous du corps ardoisé”) is not *A. cajaneus*.

- Aramides gutturalis* Sharpe, 1894. Catalogue of the Birds in the British Museum, v. 23, p. 57 and plate 5. Holotype, examined: BMNH 1843.5.24.134, “South America”. The specimen’s oldest label bears the word “Lima”. However, no species of *Aramides* is known to occur in the vicinity of Lima, Peru. If this is indeed the locality meant, then it is likely that it represents simply the port from where the skin was shipped to Europe, rather than the actual place where it was collected.
- Aramides cajanea venezuelensis* Cory, 1915. Field Museum of Natural History Ornithological Series, v. 1, n. 8, p. 296. Holotype, examined: FMNH 34472, adult male, “Encontrados, Venezuela” (Zulia state).
- Aramides cajanea peruviana* Cory, 1915. Field Museum of Natural History Ornithological Series, vol. 1, n. 8, p. 296. Holotype, examined: FMNH 44019, adult female, “Moyabamba, Peru” (San Martín department).
- Aramides cajanea latens* Bangs & Penard, 1918. Bulletin of the Museum of Comparative Zoology, v. 62, p. 41. Holotype, examined: MCZ 114297, adult female, “San Miguel Island, Bay of Panama” (known now as Isla del Rey, in the Las Perlas archipelago).
- Aramides cajanea salmoni* Chubb, 1918. Bulletin of the British Ornithologists’ Club, v. 38, p. 48. Holotype, examined: BMNH 89.11.20.50, “Remedios, Antioquia, Colombia”.
- Aramides cajanea grahami* Chubb, 1919. The Ibis, 11th series, v. 1, n. 1, p. 53. Holotype, examined: BMNH 45.8.25.56, “Pará, Brazil”.
- Aramides cajanea morrisoni* Wetmore, 1946. Proceedings of the Biological Society of Washington, v. 59, p. 50. Holotype, examined: USNM 376059, adult male, “San José Island, Archipiélago de las Perlas” (Panama).

**Diagnosis.** Nuchal spot very dark grayish-brown 10YR 3/2, sometimes duller or, very rarely, absent. Back entirely green. No white or pale feathers whatsoever on the lower chest. Basic phrase of the song bisyllabic (see details above).

**Distribution.** Pacific side of Costa Rica; Panama (including the Pearl Islands); Colombia (except the Chocó region, west of the Andes); Venezuela; the Guianas; Ecuador, Peru and Bolivia east of the Andes; Brazil (except a section of the coast where it is replaced by *A. avicenniae*, and some inland parts of the states of São Paulo, Paraná, Santa Catarina and Rio Grande do Sul, where it is replaced by *Aramides saracura*; see above); southeastern Paraguay; Uruguay; and extreme northwestern and northeastern Argentina (Jujuy, Salta, Corrientes, Entre Ríos and Buenos Aires provinces) (Figures 1 and 2).

### *Aramides avicenniae* Stotz, 1992

- Aramides cajanea avicenniae* Stotz, 1992. Bulletin of the British Ornithologists’ Club, v. 112, n. 4, p. 232. Holotype, examined: MZUSP 67212, adult male, “Iguape, São Paulo, Brazil”.

**Diagnosis.** Brown nuchal spot absent or very inconspicuous. Gray upper-back (mantle) and hindneck, with greenish-gray upper wing-coverts. No white or pale feathers whatsoever on the lower chest. Basic phrase of the song bisyllabic (see details above).

**Distribution.** Coastal Brazil from Santos, São Paulo state, south to Guaratuba Bay, Paraná state (Figures 1 and 12). A single USNM specimen from Santa Catarina state is also mentioned by Bangs (1907). According to him, it agrees completely with BMNH 89.11.20 from the Paraná coast, which we examined and is a typical *avicenni-ae*. The USNM specimen mentioned by Bangs could not be examined by us, but indicates that the species' distribution may extend further south to at least Santa Catarina.

### ***Aramides albiventris* Lawrence, 1868**

*Aramides albiventris* Lawrence, 1868. Proceedings of the Academy of Natural Sciences of Philadelphia, v. 19, p. 234. Syntypes, examined: AMNH 45656, "British Honduras" (=Belize) and AMNH 45657, "Guatemala".

*Aramides plumbeicollis* Zeledón, 1892. Anales del Instituto Físico Geográfico y del Museo Nacional de Costa Rica, tome 3, p. 134. Holotype, examined: USNM 113603, adult male, "Jiménez, lugar situado sobre la línea del ferrocarril en la planicie del Atlántico como á 56 millas del puerto de Limón, y á una altura como de 700 pies sobre el nivel del mar", Costa Rica.

*Aramides albiventris mexicanus* Bangs, 1907. The American Naturalist, v. 41, n. 483, p. 185. Holotype, examined: MCZ 110281, "Buena Vista, Vera Cruz, Mexico".

*Aramides plumbeicollis pacificus* Miller & Griscom, 1921. American Museum Novitates, n. 25, p. 11. Holotype, examined: AMNH 143684, adult male, "Tipitapa, Nicaragua".

*Aramides vanrossemi* Dickey, 1929. The Condor, v. 31, p. 33. Holotype, examined: UCLA 18750, adult male, "Barra de Santiago, Ahuachapan, El Salvador".

**Diagnosis.** Strong brown nuchal spot (Very Dark Brown 7.5YR 2.5/3). Basic phrase of the song containing at least nine notes (see above for details).

**Distribution.** From the Caribbean side of Costa Rica northwards throughout Central America to southwestern Tamaulipas state, in Mexico (Figures 1 and 2).

### **Notes on plumage variation in other species of Aramides**

#### ***Aramides ypecaha***

This species has a seemingly disjunct distribution, being found in central Brazil, especially along the Araguaia and São Francisco river valleys, as well as, further south, in southern Brazil, Paraguay, Uruguay and northeastern Argentina, but with no records from the extensive intermediate area. Nevertheless, no morphological differentiation has been described between these two populations. Based on 66 specimens, the only

difference observed was that specimens from the northern population have slightly grayer and darker backs than those from the southern population (5Y 4/3 versus 2.5Y 4/3, respectively). There is, however, considerable variation within each population, and the differences are too subtle to allow a safe, consistent diagnosis. In addition, the species' peculiar distribution needs to be further investigated before further taxonomic or evolutionary inferences can be made.

### *Aramides wolfi*

This species is considered Vulnerable in the IUCN Red List (BirdLife International 2012). It is also the *Aramides* with the most restricted distribution; only found west of the Andes from southwestern Ecuador north to the Chocó department of Colombia. From the 26 skins analyzed, we found that specimens from central and southern Ecuador are much paler than those from Colombia and the departments of Pichincha and Esmeraldas, in northern Ecuador (Figure 13). Southern specimens have pale greenish-brown backs (7.5YR 3/2 to 7.5YR 3/4), while northern ones are strong reddish-brown (5YR 2.5/2) (Figure 13). The underparts of northern specimens are also darker and redder but this is subtler than the difference in the upperparts. Where the two variants approach each other, in the region of Pichincha, Manabí and Esmeraldas, intermediates are present.

This variation coincides with a notable climatic gradient; from one of the most humid regions on Earth, in southwestern Colombia, to semi-arid conditions in southwestern Ecuador. This is consistent with Gloger's rule, according to which animal populations from humid regions tend to be darker and more pigmented than those from dry climates (Gloger 1833, Zink and Remsen 1986). The mechanisms behind Gloger's rule are not necessarily genetic (Zink and Remsen 1986, and see Beebe 1907, Slagsvold and Lifjeld 1985), and thus we refrain from making any taxonomic or evolutionary inferences based on the variation observed in *A. wolfi*, and suggest that further investigations are required to determine the mechanisms responsible for it and the taxonomic implications thereof.

### *Aramides mangle*

This species occurs along the coast of Brazil from Pará to Paraná, with some inland records in northeastern Brazil which indicate occurrence of migratory movements (Redies 2010, Marcondes et al. 2014). Two plumage variants were observed in it. The coloration pattern is the same in the two, but in one variant the whole plumage is much paler. Even though pale specimens come mainly from northeastern Brazil, there is no geographical segregation between the variants, as dark-plumaged birds also occur in that region (e. g. FMNH 403199, from Piauí, and MPEG 67808, from Maranhão). Indeed both forms have even been collected in the same locality (MNHN 1971.786 and 1971.787, from Exu, Pernambuco). Given this lack of geographical pattern, the plumage variation in *Aramides mangle* is considered intraspecific and taxonomically uninformative. Its exact nature remains uncertain, but we hypothesize either that (1) the pale individuals are juveniles, although there are no notes on any of the specimen



**Figure 13.** A series of *Aramides wolfi* in the AMNH. The six leftmost specimens, with a stronger coloration, are from northwestern Ecuador, while the four specimens to the right, from southwestern Ecuador, have a paler plumage.

labels regarding their age. (2) Dark and paler specimens represent an intraspecific polymorphism with two distinct, discrete plumage morphs or phases. Or (3) that paleness is due to feather wear, possibly related to abrasion or exposure to sunlight.

## Acknowledgements

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## **Supplementary material 1**

### **Specimens examined**

Authors: Rafael Sobral Marcondes

Data type: measurement

Explanation note: A list of specimens (skins) examined of this study.

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## **Supplementary material 2**

### **Recordings examined**

Authors: Rafael Sobral Marcondes

Data type: tape recording

Explanation note: A list of tape recordings examined for this study.

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# Can whole-drawer images measure up? A reply to Johnson et al. (2013)?

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## Abstract

Johnson et al. (2013) found that morphometric measurements of dragonfly wings taken from actual specimens and measurements taken from whole-drawer images of those specimens were equally accurate. We do not believe that their conclusions are justified by their data and analysis. Our reasons are, first, that their study was constrained in ways that restrict the generalisability of their results, but second, and of far greater significance, their statistical approach was entirely unsuited to their data and their results misled them to erroneous conclusions. We offer an alternative analysis of their data as published. Our reanalysis demonstrates, *contra* Johnson et al., that measurements from scanned images are not a reliable substitute for direct measurement.

## Keywords

Digitization, entomological collections, morphometrics, museum collections, dragonflies, Odonata

## Introduction

The use of whole-drawer imaging to rapidly digitize insect collections has been promoted in a recent special issue of this journal (#209, 2012). While various imaging technologies have been used (Blagoderov et al. 2012; Mantle et al. 2012; Bertone et al. 2012; Dietrich et al. 2012; Schmidt et al. 2012), all have the advantage of providing

rapid digital access to the specimen holdings of entomological natural history collections. Remote curation is then possible, provided single dorsal specimen images are sufficient for identification. However, while whole-drawer imaging is a form of digitization, it is not a substitute for specimen databasing, and by itself produces images of groups of specimens that are not databased. Significant disadvantages to the method are (1) that the drawer images are not easily associated with the geocoded specimen data from the specimens contained in the drawers, and (2) the images represent a snapshot in time that will change when the drawers are curated and/or any specimens are added, removed or moved in the drawer. Most often label data is partly or entirely obscured by the insect above, further reducing the utility of whole-drawer images in specimen databasing initiatives. In addition, some of the imaging methods produce images with distortion and curvature around their edges.

We have no doubt that some of the challenges of using Satscan images in the curation of insect collections will be overcome by future technological and workflow improvements; however, we agree with Ang et al. (2013) that digitization efforts should only proceed if they enhance the quality and quantity of taxonomy, are feasible and have favourable cost-benefit ratios. For example, many, perhaps 20, expert international curation and research visits could be arranged for the equivalent cost of a Satscan device used in some collections for whole-drawer imaging, and many similar curation and research visits could be arranged for the same cost as the annual service contract and ongoing operational labour inputs.

Whole-drawer images could possibly be used for extracting morphometric measurements from the insects in the drawers, increasing their value in addressing scientific questions including taxonomic ones. In a recent edition of this journal, Johnson et al. (2013) compared three methods for taking morphometric measurements, specifically of wing length, from museum specimens of pinned and set insects. Their conclusion was that measurements taken from actual insects and those taken from whole-drawer images of specimens were equally accurate. Our reanalysis of their data, however, suggests that measurements from scanned images are not a reliable substitute for direct measurement.

It is generally accepted, in entomological collection practice, that the most accurate method for taking morphological measurements of a pinned insect is to excise the body part from the specimen, mount it on a microscope slide, and then measure it using a calibrated eyepiece or other micrometer. The advantage of slide mounting is that the body part is held flat and at the proper angle for taking the measurement. On the downside, slide mounting is a slow and resource-intensive process. Its greatest disadvantage, though, is that the specimen must be damaged if not destroyed. This disadvantage makes the slide-mount method unsuitable in many instances.

A quicker, and non-destructive method is to take measurements *in situ* using hand-held calipers. This usually involves temporarily removing the pinned specimen from its drawer and orienting it so the part to be measured is open to view. In modern practice the measurement typically is taken with a set of fine-tipped digital calipers. The advantages of this caliper method over the slide-mount method are that measurements are easier to take and the specimen need not be damaged. The perceived disadvantages are that a

hand-held measurement may be less accurate and/or less repeatable than a slide-mount measurement, and that results might vary depending on who takes the measurements.

In recent years a new non-destructive method has become available. Digital scanning technology is now such that an undistorted, evenly-scaled digital images can be taken of an entire drawer of pinned insects (Beaman and Cellinese 2012). In principle, morphometric measurements could be taken from the scan. An obvious advantage of this method is that an entire collection could be scanned and the images held on file. The chief theoretical disadvantage is that lengths as measured from the scanned image might be on average too short, the issue being that unless the part to be measured is oriented precisely in the plane of the camera it will appear foreshortened in the image.

Johnson et al. set out to test whether the caliper method and scan method are acceptable alternatives to the slow, difficult and destructive slide-mount method. They measured the lengths of the right forewings in each of 71 pinned specimens of Odonata (dragonflies and damselflies), using first the scan method, then the caliper method, and finally the slide-mount method. Each wing was measured three times by each method. The same operator took all 639 measurements. Johnson et al. in fact ran two variants of the slide-mount method. In the first each slide was labeled with its specimen number. In the second the label was replaced with a randomly assigned code. The results did not differ. For simplicity we refer to the second version only. Nothing in our conclusions would be altered if the first version was used instead.

Johnson et al. calculated the average wing length of their sample insects under each method. The slide-mount method gave this average as 29.24 mm, the caliper method gave 29.38 mm and the scan method gave 28.77 mm. They calculated the standard error of each estimate. It was 1.04 mm under any method. Two correlation coefficients also were calculated, the first was between lengths estimated by the caliper method and the slide-mount method, the second was between lengths estimated by the scan method and the slide-mount method. These two correlation coefficients were then compared.

Johnson et al. argue that although the caliper method overstates the average length by 0.14 mm and the scan method understates it by 0.47 mm, each estimate lies within one standard error of the average length from the slide-mount method, and so each alternative method gives an acceptable measure of length. Likewise, there being no significant difference between the two correlation coefficients, they argue that both the caliper and the scan methods are equally accurate.

Why do we not accept these conclusions? Two relatively minor issues can be dealt with briefly. First, while a major concern with the caliper method is that it may lack repeatability across different practitioners, Johnson et al. did not address this issue. They showed only that one particular practitioner overestimated wing lengths by an average 0.14 mm. This single data point tells us very little. The study would need to be repeated several times by different practitioners before any general conclusion could be drawn.

Second, on examining their data on repeat measures within the scan method we observed a pattern that suggests a possible problem. We enquired of the corresponding

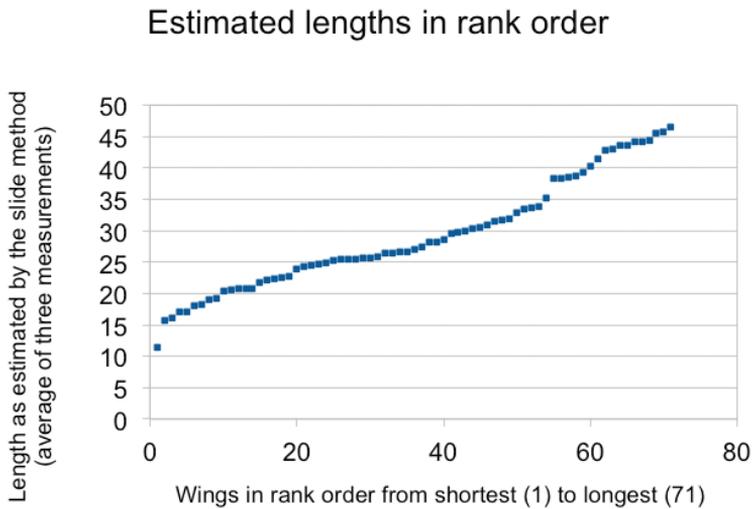
author, and it transpires the scan method was not fully replicated. The scan was taken only once, with measurements being taken three times from the same image. Thus, Johnson et al. understate the variability or overestimate the repeatability of this method by leaving out measurement error associated with making the scan.

Our chief reason, however, for rejecting the conclusions that Johnson et al. came to, is not about these issues but instead concerns the statistical approach they took when analyzing their data. They applied statistical methods which would be appropriate only if every measurement were of an average-length wing and the only source of length variation across the sample was measurement error, a proposition patently not true of their data. From personal knowledge of the drawers of specimens on which their study was based, their specimens range in size from *Nannophya dalei* with wing length about 11 mm, to *Hemianax papuensis* at 47 mm. A majority of their specimens were from species of moderate size, say between 25 and 35 mm forewing length, but the average of all lengths in their sample refers to no species at all. The standard error of the estimate of an average length, the 1.04 mm which Johnson et al. use as their standard against which to judge the performance of the methods, is largely a result of some wings being long and others short. It has very little to do with measurement error. It is illogical to say, of these data, that a measurement method should be regarded as acceptable if it can produce an average wing length that lies within 1.04 mm (or should that be 2.08 mm?) of the true value. Johnson et al. make a similar error with the correlation coefficients. It should be no surprise that the correlations are similar, because no method is so poor that it mistakes a small wing for a large one. That their two correlation coefficients are not dissimilar in a standard statistical test for the difference between two correlation coefficients is almost entirely due to the sampled wings being of different sizes. Nothing about the efficacy of the measurement methods can be inferred from that statistic.

## Reanalysis

Fortunately, Johnson et al. followed good practice and published their raw data in full. The analysis that follows takes the approach that comparisons ought to be made pairwise, wing by wing. The basic approach is that the three repeat measurements for each wing under each measurement method are averaged, and those three sets, each of 71 length estimates, are compared. We proceed by way of three related figures (Figures 1–3). In each figure the horizontal axis shows the 71 specimens arranged in size from small to large according to the slide-mount method.

The vertical axis in Figure 1 shows wing length. The message is that we have reasonable coverage of wings in the size range 15–45 mm. The several short ‘runs’ comprising a few wings of nearly identical size each represent, we may be fairly sure, one species. The vertical ‘gaps’, such as between 35 mm and 38 mm, represent lengths that are not sampled, quite possibly because no dragonflies in that size range occur where these specimens were collected.

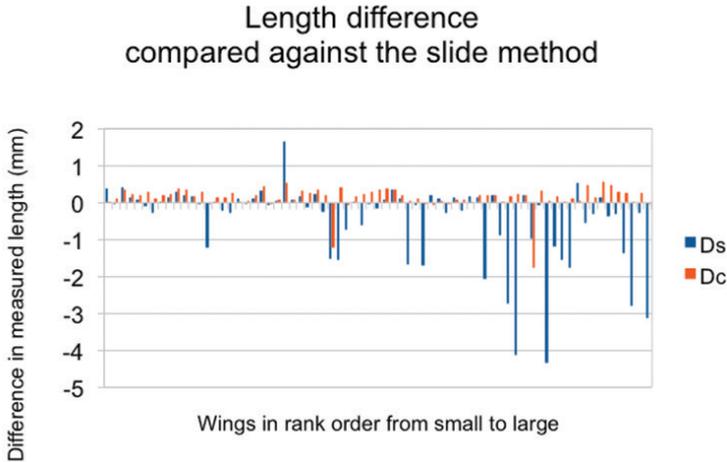


**Figure 1.** Averages (across the three repeat measurements) by the slide-mount method. Wings are arranged in size order.

Rather than compute the length of an ‘average’ wing, which is a biologically meaningless use of these data, let us note that the aggregate of all 71 measured lengths (averaged across the three repeat measurements) is 2076 mm by the slide-mount method, 2086 mm by the caliper method, and 2043 mm by the scan method. In other words, the caliper method, on average, has overstated the lengths by 0.48% (0.14 mm) while the scan method has understated them by 1.61% (0.47 mm) (using the slide-mount estimates as a reference length). These averaged differences or biases among the methods are, of course, exactly as reported by Johnson et al.

We might surmise that bias when using the caliper method might tend towards a fixed quantity that is independent of wing length. That would happen if the zero point of the calipers was wrongly set or the practitioner tended always to hold the instrument in some particular way that did not line up the instrument exactly with the specimen. Likewise, we might surmise that measurement bias in the scan method would tend towards a constant proportion. A constant percentage error would be expected, on averaging across many specimens, if the bias resulted primarily from some wings not being set in the horizontal plane.

Figure 2 shows differences in wing length;  $D_c$  represents the differences in length (averaged across three repeat measurements) between the caliper method and the slide-mount method,  $D_s$  represents the differences in length (similarly averaged) between the scan method and the slide-mount method. The differences in the first series are, indeed, quite uniformly distributed across all sizes of wing. Two large negative outliers, -1.22 mm (ranked data point 30, specimen JT63) and -1.77 mm (ranked data point 56, specimen JT60), drag the average down. Without access to the raw score sheets those scores cannot be verified but they look a lot like recording errors. On removing



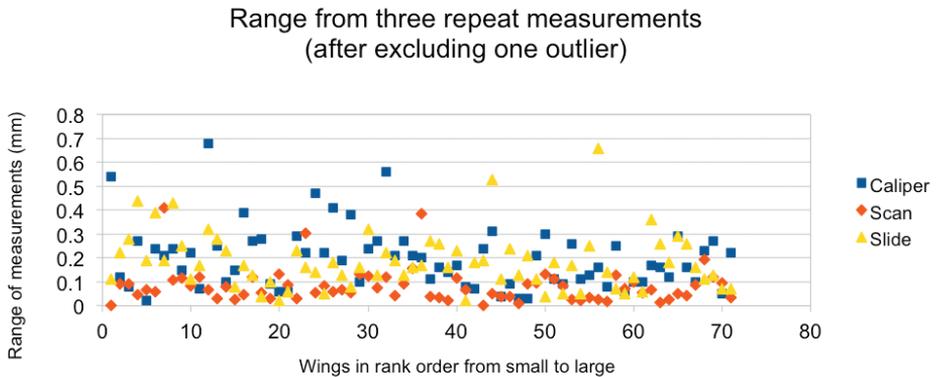
**Figure 2.** Averaged (across the three repeat measurements) length differences between pairs of methods. The order of the specimens is the same as for Figure 1. One series (Dc) is of differences between slide-mount and caliper lengths, the other (Ds) is between slide-mount and scan method lengths.

them from the calculation the average bias of the caliper method increases from +0.14 mm to +0.19 mm (+0.65%).

The second series (Ds) shows a pattern of frequent very large negative differences concentrated almost entirely in the large-winged half of the sample. This is in line with expectations if the main source of measurement error is foreshortening that affects some but not all specimens. One large positive outlier at ranked data point 24 (specimen JT33) does not fit any foreshortening explanation, and another outlier, ranked data point 14 (specimen JT20) appears very short in percentage terms and may also need separate explanation. As the figures stand, though, the scan method has understated the length in each of twenty specimens, being 28% of the sample, by more than 0.5 mm. The greatest difference, at -4.35 mm, is for ranked data point 58 (specimen JT19).

While this downward bias, expressed in absolute length difference, is greater for longer wings, long wings also show a higher proportionate bias. A least squares regression through the Ds scores (re-expressed as a percentage of wing length), and wing lengths by the slide-scan method, has a downward slope of 0.14% per millimetre of wing. This bias over and above what might be expected from foreshortening alone is explicable if, as is suggested by the Figure 2, a greater proportion of long wings than short wings are not exactly at right angles to the scanner. The average bias in the scan method as calculated from the regression would be close to 4% for a 46 mm wing. This average does not mean much, though, when in wings of every length the bias is concentrated in particular specimens.

It remains to examine each method for its repeatability. The ranges of the three repeat measurements can be used as an indicator. Fig. 3 shows the range of the three measurements, in mm, for each wing by each method.



**Figure 3.** The range, in mm, for each wing by each method, specimens order being the same as before. Green symbols refer to the slide-mount method, blue to the caliper method and red to the scan method. One extreme outlier (ranked data point 42, specimen JT69) was removed.

There are no apparent trends in these intra-method repeatability statistics associated with wing size. Taking an average across the 71 observations, the slide-mount and caliper methods perform equally well. Averaged ranges are 0.185 mm (s.e. 0.119 mm) and 0.197 mm (s.e. 0.130 mm) respectively. The difference between the observed means is not significant. At first sight the scan method appears to be more repeatable than either the caliper or the slide-mount method. The average range within the scan method is 0.083 mm after removing an outlier not shown in the figure (ranked data point 42, specimen JT69, range 2.03 mm). However, as established earlier, these data omit any error associated with repeating the scan, and so the comparison with the other methods is incomplete.

## Discussion and conclusion

Wings of various lengths within the range 11–47 mm have been measured by three methods, with sufficient coverage between 15 mm and 45 mm to give results that should be applicable within that range. The slide-mount method has been taken as a benchmark against which to compare the caliper method and the scan method. The sample (this from personal knowledge) was of typical drawers of pinned Odonata set by competent entomologists. The specimens were not of ‘show’ quality but neither were they of inferior quality. They were of a standard typically found in museum collections.

Using the caliper method, one practitioner has overestimated wing lengths by, on average, 0.19 mm. This bias was constant across the size range. The repeatability of the caliper method was similar to that of the slide-mount method, and the differences among repeat measurements are of similar size to the bias between the two methods.

Further studies are needed to examine whether this bias and these levels of intra-method repeatability apply more broadly to other practitioners.

Using the scan method, the same practitioner underestimated wing lengths by, on average 0.47 mm. These errors were not constant across all wing sizes, and neither did they appear in all specimens. They were distributed erratically amongst some 28% of specimens and large errors, though not large percentage errors, occur almost exclusively in the long-winged half of the sample. Some of the errors were enormous; to >0.4 cm (and >10% of wing length) in the extreme case. The repeatability of this method has yet to be adequately examined.

The pattern of errors within the scan method contrasts with errors made by the same practitioner using the other methods. This suggests it is not an operator effect, and the limited information we have about scan measurement repeatability (which says it is similar to slide-mount and caliper repeatability only lower) confirms that conclusion. Rather, this pattern of errors is as would be expected under the hypothesis that downward bias occurs whenever a wing lies at an angle so that the image is foreshortened.

That this bias should apply to some 28% of specimens, and indeed to more than 40% of wings longer than about 25 mm in length, should be cause for concern. These data strongly suggest that the scan method is not suitable for use on larger insects. A method that can under-estimate in excess of 25% of wing lengths by more than a half millimetre, and at times produce errors of almost half a centimetre, is surely of little value as a measurement tool for entomologists. The method is not suitable for use on smaller insects either, because while a majority of wing lengths might be slightly underestimated, an occasional wing still is grossly underestimated by this method.

That said, if the technology of scanning could be improved to the point where out-of-plane wings could be recognised as such and the appropriate trigonometric corrections applied to measurements of the scanned image, the scan method might yet prove to contain an alternative to the other two methods.

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