Twenty years after: What larval characters are still telling us about the phylogeny of frogs (Amphibia: Anura)

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Zoologisches Museum Hamburg & UNC Charlotte

XLI Annual Meeting of the Willi Hennig Society

PHdSD, AH: Leibniz Institut Zur Analyse Des Biodiversitätswandels, Zoologisches Museum Hamburg, Zentrum Für Taxonomie Und Morphologie.
DJM: UNC Charlotte, Dept. of Bioinformatics and Genomics &
CIPHER Research Center.

Muito obrigado!

¡Muchas gracias!

Thank you!

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LIB Leibniz-Institut zur Analyse des Biodiversitätswandels

MARIE CURIE ACTIONS

UNIVERSITY OF NORTH CAROLINA CHARLOTTE

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CENTER FOR COMPUTATIONAL INTELLIGENCE TO PREDICT HEALTH AND ENVIRONMENTAL RISKS

XLI ANNUAL MEETING OF THE WILLI HENNIG SOCIETY
JULY 28 - AUGUST 1, 2024 • INSTITUTO DE BIOLOGÍA, UNAM, MEXICO CITY

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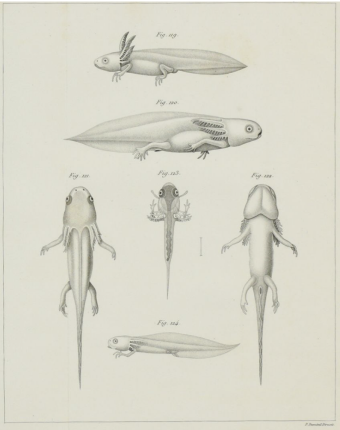
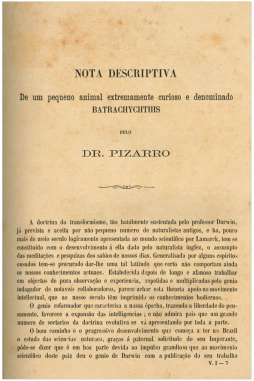
Antoine Dugès (1834) and William Parker (1871, 1876, 1881)

- Dugès (1834) described the first anuran chondrocranium while studying the development of the musculoskeletal system in amphibians.
- William K. Parker published a series of monographs on the vertebrate skull (*e.g.*, 1871, 1876, 1881) and included detailed descriptions of the chondrocranium of several anurans, such as *Pseudis* and *Rana* tadpoles.



Pizarro (1876)

One of the first taxonomical and systematic papers using larval morphology as evidence was that of **Pizarro** (1876), who proposed the erection of the genus *Batrachychthis* to the weird-looking tadpole of *Pseudis platensis*.



NOTA DESCRIPTIVA

De um pequeno animal extremamente curioso e denominado
BATRACHYCHTHIS

PELO

DR. PIZARRO

A doctrina do transformismo, tão habilmente sustentada pelo professor Darwin, já prevista e aceita por não pequeno numero de naturalistas antigos, e ha, pouco mais de meio seculo logicamente apresentada ao mundo scientifico por Lamarck, tem se constituido com o desenvolvimento á ella dado pelo naturalista inglez, o assumpto das meditações e pesquizas dos sabios de nossos dias. Generalisada por alguns espiritos ousados tem-se procurado dar-lhe uma tal latitude que certo não comportam ainda os nossos conhecimentos actuaes. Estabelecida depois de longo e afanoso trabalhar em objectos de pura observação e experiencia, repetidas e multiplicadas pelo genio indagador de notaveis collaboradores, parece achar esta theoria apoio no movimento intellectual, que ao nosso seculo têm imprimido os conhecimentos hodiernos.

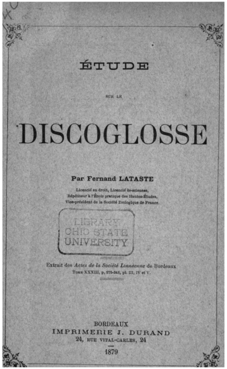
O genio reformador que caracterisa a nossa épocha, trazendo a liberdade do pensamento, favorece a expansão das intelligencias ; e não admira pois que um grande numero de sectarios da doctrina evolutiva se vá apresentando por toda a parte.

O bom caminho e o progressivo desenvolvimento que começa a ter no Brazil o estudo das sciencias naturaes, graças á paternal solicitude do seu Imperante, póde-se dizer que é em boa parte devido ao impulso grandioso que ao movimento scientifico deste paiz deu o genio de Darwin com a publicação do seu trabalho

V. I — 7

Lataste (1879)

French zoologist Fernand **Lataste** was the first one to use larval characters in the anuran systematic. He used the position of the spiracle to propose an ordinal classification of frogs. He suggested the groups Laevogyrinidae, for the frogs with a left spiracle, and Mediogyrinidae for frogs with a medial spiracle.


ÉTUDE

SUR LE

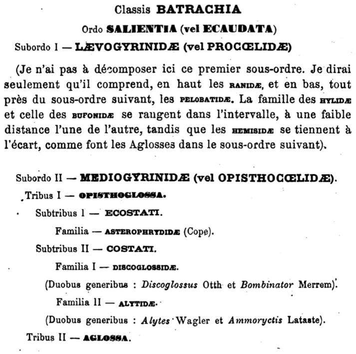
DISCOGLOSSE

Par Fernand LATASTE

Licencié en droit, Licencié ès-sciences,
Répétiteur à l'École pratique des Hautes-Études,
Vice-président de la Société Zoologique de France.

Extrait des *Actes de la Société Linnéenne* de Bordeaux
Tome XXXIII, p. 275-344, pl. III, IV et V.

BORDEAUX
IMPRIMERIE J. DURAND
24, RUE VITAL-CARLES, 24
1879


Classis **BATRACHIA**

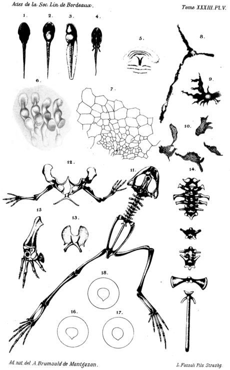
Ordo **SALIENTIA (vel ECAUDATA)**

Subordo I — **LÆVOGYRINIDÆ (vel PROCŒLIDÆ)**

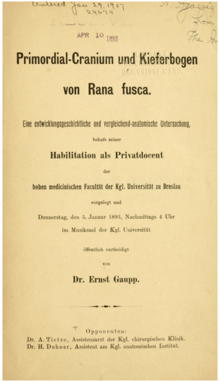
(Je n'ai pas à décomposer ici ce premier sous-ordre. Je dirai seulement qu'il comprend, en haut les RANIDÆ, et en bas, tout près du sous-ordre suivant, les PELOBATIDÆ. La famille des HYLIDÆ et celle des BUFONIDÆ se rangent dans l'intervalle, à une faible distance l'une de l'autre, tandis que les HEMISIDÆ se tiennent à l'écart, comme font les Aglosses dans le sous-ordre suivant).

Subordo II — **MEDIOGYRINIDÆ (vel OPISTHOCŒLIDÆ).**

- Tribus I — **OPISTHOGLOSSA.**
  - Subtribus I — **ECOSTATI.**
    - Familia — **ASTEROPHRYDIDÆ** (Cope).
  - Subtribus II — **COSTATI.**
    - Familia I — **DISCOGLOSSIDÆ.**
      (Duobus generibus : *Discoglossus* Otth et *Bombinator* Merrem).
    - Familia II — **ALYTIDÆ.**
      (Duobus generibus : *Alytes* Wagler et *Ammoryctis* Lataste).
- Tribus II — **AGLOSSA.**



Ernst Gaupp (1893)



Entered Jan 29, 1907
28674

N. Agassiz
from
The Au

APR 10 1893

Primordial-Cranium und Kieferbogen

von Rana fusca.

Eine entwicklungsgeschichtliche und vergleichend-anatomische Untersuchung,

behufs seiner

Habilitation als Privatdocent

der

hohen medicinischen Facultät der Kgl. Universität zu Breslau

vorgelegt und

Donnerstag, den 5. Januar 1893, Nachmittags 4 Uhr

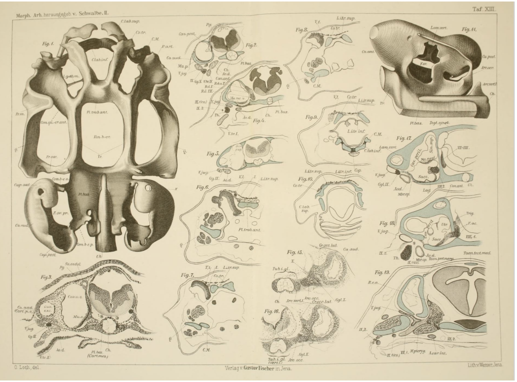
im Musiksaal der Kgl. Universität

öffentlich vertheidigt

von

Dr. Ernst Gaupp.

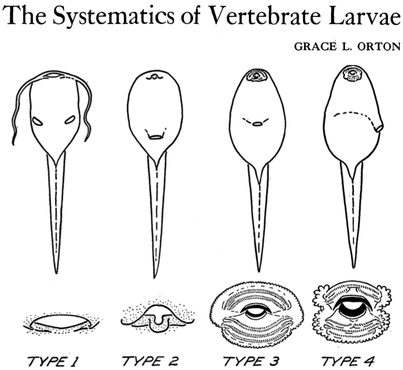
Opponenten:
Dr. A. Tietze, Assistenzarzt der Kgl. chirurgischen Klinik.
Dr. H. Dehner, Assistent am Kgl. anatomischen Institut.



Shout-out to Ernst Gaupp (1893), who studied the chondrocranium of *Rana temporaria* and launched a very elaborate terminology.

Grace L. Orton (1953)

Grace L. **Orton** (1953) was revolutionary to propose that frogs should be grouped into four major lineages according to their tadpole types. **Type 1** would lack mouthparts and possess a paired ventral spiracle; **Type 2** would also lack mouthparts but have a single, medial spiracle; **Type 3** tadpoles have mouthparts, but a single, medial spiracle; **Type 4** have mouthparts and a lateral spiracle.



The Systematics of Vertebrate Larvae

GRACE L. ORTON

Grace L. Orton (1953)

"For many years, studies on early developmental stages were guided by the idea that larval characters have an ancestral, recapitulative significance, or by the belief that they are secondary adaptations with little or no evolutionary importance. Neither theory is adequate from the standpoint of systematics, and together they have somewhat hampered effective use of larval data by taxonomists" (p. 63).

Kluge & Farris (1969)

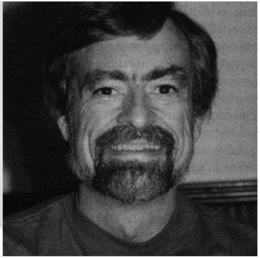
Kluge & Farris (1969) started the quantitative phylogenetic era with a seminal paper, which included a character about the spiracle morphology. Also, they dedicated several lines to discuss the homology and evolution of that system.

QUANTITATIVE PHYLETICS AND
THE EVOLUTION OF ANURANS

ARNOLD G. KLUGE AND JAMES S. FARRIS

1969

Arnold Kluge

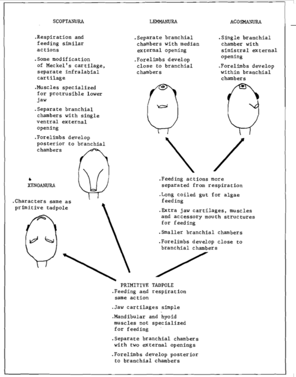


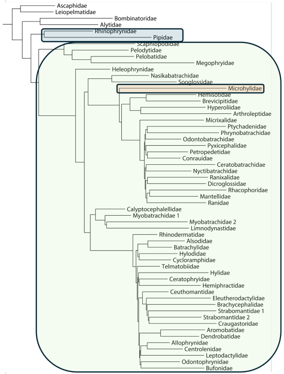
James S. Farris

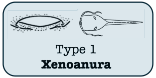
1969 was when the Apollo 11 landed on the moon.

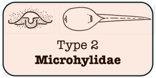
Starrett (1973)

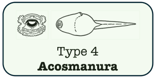
Later, **Starrett** (1973) would formally name tadpole's types as Xenoanura, Scoptanura, Lemanura, and Acosmanura. Some of these are still naming (partially) monophyletic clades nowadays.







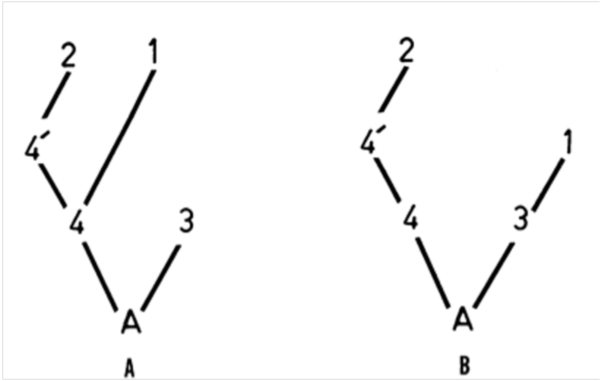




Topology from Portik *et al.* (2023).

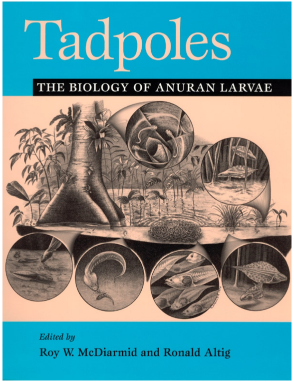
Sokol (1975)

Sokol revised the classification of Orton and Starrett and suggested that type 1 tadpoles were not the most “basal” ones but instead highly modified.



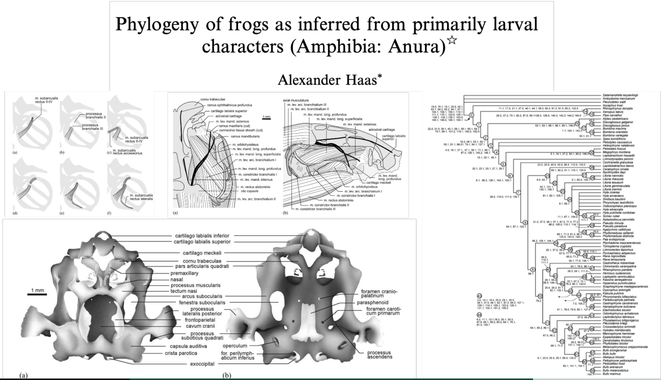
Cannatella (1999)

By the way, the aforementioned works were compiled by Cannatella (1999), in his review of the musculoskeletal system published in the book “Tadpoles.”



Phylogeny of frogs as inferred from primarily larval characters

Haas (2003) performed the most comprehensive study about larval morphology and evolution. He scored 141 phenotypic characters (mainly from the muscles and skeleton) for 85 amphibians species. In that study he was able to antecipate many phylogenetic and taxonomic patterns later recovered in large-scale molecular studies (*e.g.*, Frost *et al.* 2006, Pyron and Wiens 2011; Jetz & Pyron 2018). Also, he proposed several novel synapomorphies at different levels of inclusiveness.

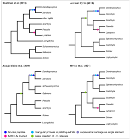


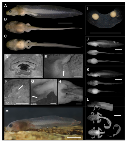
Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura)☆

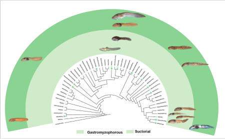
Alexander Haas*

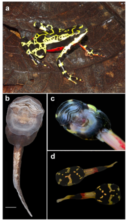
What is going on since Haas (2003)

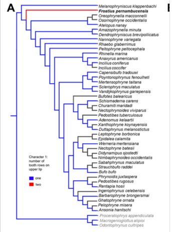
Since Haas (2003), many authors discussed characters evolution, but information is scattered in the literature and focused on particular taxa. Nevertheless, many of those studies proposed novel synapomorphies, increasing the bulk of evidence that larval morphology can contribute positively for our understanding of phylogenetic relationships.

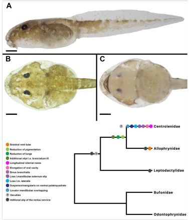






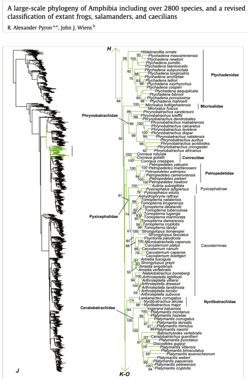






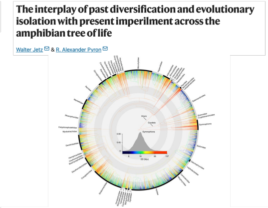
What is going on since Haas (2003)

Frogs phylogeny, however, have been largely dominated by molecular studies (*e.g.*, Pyron & Wiens 2011; Feng *et al.* 2017; Jetz & Pyron 2018; Portik *et al.* 2023) and phenotypic evidence have been largely neglected.



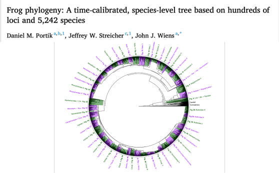
A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians

R. Alexander Pyron[a,*], John J. Wiens[b]



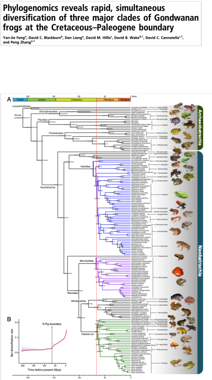
The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life

Walter Jetz & R. Alexander Pyron



Frog phylogeny: A time-calibrated, species-level tree based on hundreds of loci and 5,242 species

Daniel M. Portik[a,b,1], Jeffrey W. Streicher[c,1], John J. Wiens[a,*]

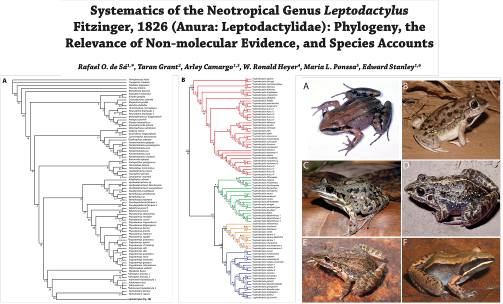


Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary

Yan-Jie Feng, David C. Blackburn, Dan Liang, David M. Hillis, David B. Wake, David C. Cannatella, and Peng Zhang

What is going on since Haas (2003)

This is highly paradoxical, as many studies demonstrated the positive impact of phenotypic data in phylogenetic studies. For instance, **de Sá** *et al.* (2014) performed a separate analysis of partionated data. In the molecular analysis, *Hydrolatere* was recovered and nested within *Leptodactylus*. When phenotypic characters were included, the recovered *Leptodactylus* as monophyletic, even though morphology was not scored for *Hydrolatere*. Aside from that, relative branch support increased.

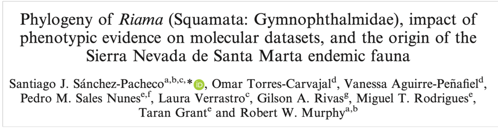


Systematics of the Neotropical Genus *Leptodactylus* Fitzinger, 1826 (Anura: Leptodactylidae): Phylogeny, the Relevance of Non-molecular Evidence, and Species Accounts

Rafael O. de Sá[1,], Taran Grant[2], Arley Camargo[1,3], W. Ronald Heyer[4], Maria L. Ponssa[5], Edward Stanley[1,6]*

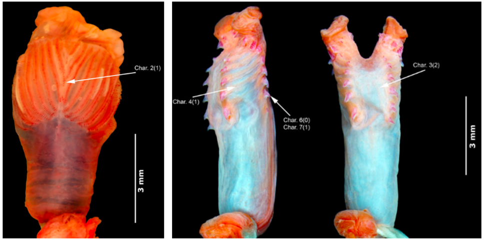
What is going on since Haas (2003)

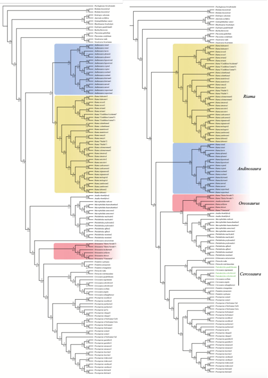
Similarly, **Sanchez-Pacheco** *et al.* (2018) recovered very different topologies with and without morphology, which also increased branch support when added.



Phylogeny of *Riama* (Squamata: Gymnophthalmidae), impact of phenotypic evidence on molecular datasets, and the origin of the Sierra Nevada de Santa Marta endemic fauna

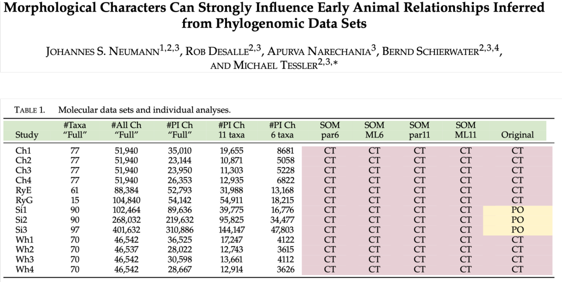
Santiago J. Sánchez-Pacheco[a,b,c,*], Omar Torres-Carvajal[d], Vanessa Aguirre-Peñafiel[d], Pedro M. Sales Nunes[e,f], Laura Verrastro[c], Gilson A. Rivas[g], Miguel T. Rodrigues[e], Taran Grant[e] and Robert W. Murphy[a,b]





What is going on since Haas (2003)

More impressive, **Neumann** *et al.* (2021) showed that in a genomic study with up to 400,000 molecular characters, the inclusion of as much as 275 phenotypic characters was able to change the sister group to all metazoans (SOM).

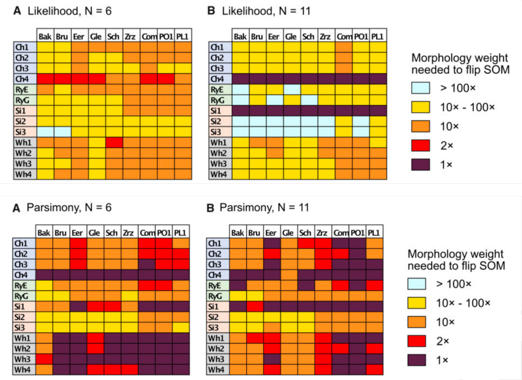


Morphological Characters Can Strongly Influence Early Animal Relationships Inferred from Phylogenomic Data Sets

JOHANNES S. NEUMANN[1,2,3], ROB DESALLE[2,3], APURVA NARECHANIA[3], BERND SCHIERWATER[2,3,4], AND MICHAEL TESSLER[2,3,*]

TABLE 1. Molecular data sets and individual analyses.

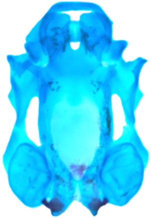
Study	#Taxa "Full"	#All Ch "Full"	#PI Ch "Full"	#PI Ch 11 taxa	#PI Ch 6 taxa	SOM par6	SOM ML6	SOM par11	SOM ML11	Original
Ch1	77	51,940	35,010	19,655	8681	CT	CT	CT	CT	CT
Ch2	77	51,940	23,144	10,871	5058	CT	CT	CT	CT	CT
Ch3	77	51,940	23,950	11,303	5228	CT	CT	CT	CT	CT
Ch4	77	51,940	26,353	12,935	6822	CT	CT	CT	CT	CT
RyE	61	88,384	52,793	31,988	13,168	CT	CT	CT	CT	CT
RyG	15	104,840	54,142	54,911	18,215	CT	CT	CT	CT	CT
Si1	90	102,464	89,636	39,775	16,776	CT	CT	CT	CT	PO
Si2	90	268,032	219,632	95,825	34,477	CT	CT	CT	CT	PO
Si3	97	401,632	310,886	144,147	47,803	CT	CT	CT	CT	PO
Wh1	70	46,542	36,525	17,247	4122	CT	CT	CT	CT	CT
Wh2	70	46,537	28,022	12,743	3615	CT	CT	CT	CT	CT
Wh3	70	46,542	30,598	13,661	4112	CT	CT	CT	CT	CT
Wh4	70	46,542	28,667	12,914	3626	CT	CT	CT	CT	CT



The impact of phenotypic evidence in largely DNA-dominated matrixes

- An increasing body of evidence suggests that phenotypic characters can improve clade resolution, affect the positioning of specific taxa, and increase the relative branch support of phylogenetic hypotheses.
    - For example, Haas (2003) anticipated several phylogenetic trends that the following generation of large-scale molecular studies has since supported.
- Moreover, fossils can only be placed in trees if morphological characters are included.

Twenty years after



- Here, we revisited Haas' work and compiled all the literature information published after 2003.
  - We also analyzed several other tadpoles.
- Our goals are twofold:
  1. To test the impact of larval morphology on largely DNA-dominated matrices; and
  2. To summarize the phenotypic variation in larval morphology reported in the last 20 years in a phylogenetic framework.

Choice of phylogenetic method

My personal view on total evidence:

- "We consider the results of the total evidence analysis to be the phylogenetic hypothesis that best explains the evidence, because the analysis of all available evidence maximizes explanatory power (Kluge, 1989; Grant and Kluge, 2003), and all discussion of character evolution and phenotypic synapomorphies is based on the results of total evidence analysis" (Araujo-Vieira *et al*. (2019), Cladistics 35: 469-486, p. 472)

Choice of phylogenetic method

My personal view on weighted vs. unweighted parsimony:

- Phylogenetics minimizes the character transformations needed to explain the observations.
- Unweighted (equally weighted) parsimony analysis minimizes hypothesized transformations globally (this follows a "Kluge-an" reasoning, of course).
- The assumptions (expressed as differential probabilities or costs) about the evolutionary process or perceived importance of different classes of transformations employed in maximum likelihood, Bayesian inference, and weighted parsimony methods minimize certain classes of transformations at the expense of others (and I present this as an observation, not a criticism).
- Operational considerations aside (*e.g.*, treespace searching capabilities), disagreements between the results of unweighted parsimony analysis and the other methods are due to the increased patristic distance required to accommodate the additional assumptions (which is a "Grant-ean" point of view).

Choice of phylogenetic method

To sum up:

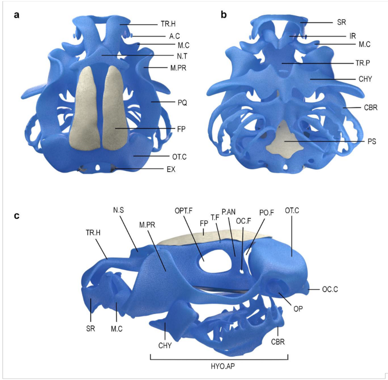
- Here, we use unweighted parsimony for a total evidence analysis.
- An advantage is that thorough analysis can be done “fast” (not that weighted parsimony is much slower), and I find this approach much clearer and straightforward.
- Also, I need a few more months to understand Phylogenetic Minimum Description Length (PMDL) and become proficient in PhyG before I can confidently use PMDL instead of parsimony.

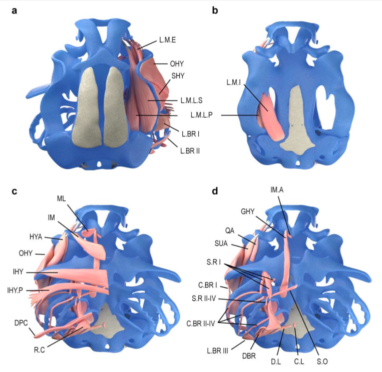
Data overview (detail explanations next)

- The starting phenotypic dataset contains 282 taxa and 137 phenotypic characters individualized from:
  - external morphology,
  - musculoskeletal system,
  - and visceral components of tadpoles.
- Additionally, we included up to 15 molecular markers, summing up to 13,982 characters for 206 taxa.
  - 5 mitochondrial: MT-CYB, MT-ND1, MT-ND2, MT-RNR1 (12S rRNA), and MT-RNR2 (16S rRNA)
  - 10 nuclear: BDNF, CXCR4, H3C1, POMC, RAG1, RHO, SIAH1, SLC8A1, SLC8A3, and TYR
    - Nervous functions: BDNF (Brain-derived neurotrophic factor), H3C1 (H3A histone), and POMC (Pro-opiomelanocortin)
    - Immune functions: RAG1
    - Cellular function: CXCR4, RHO, SIAH1, and SLC8A1 (NCX1)
    - Pigmentation: TYR (Tyrosinase) and SLC8A3

Vera Candioti, Dias, and Haas (*in press*)

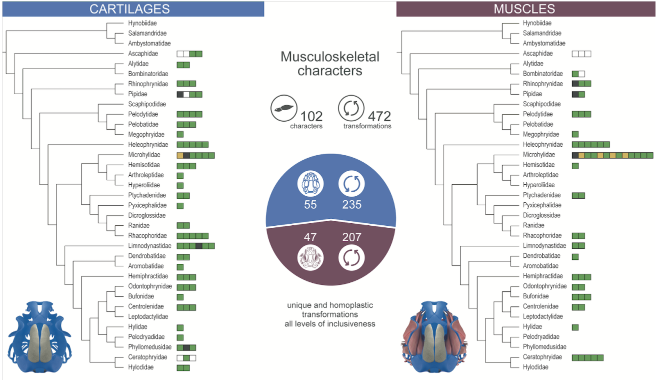
Recently, **Vera Candioti**, Dias, and Haas did a review about the muscleskeleton system in the anuran larvae.



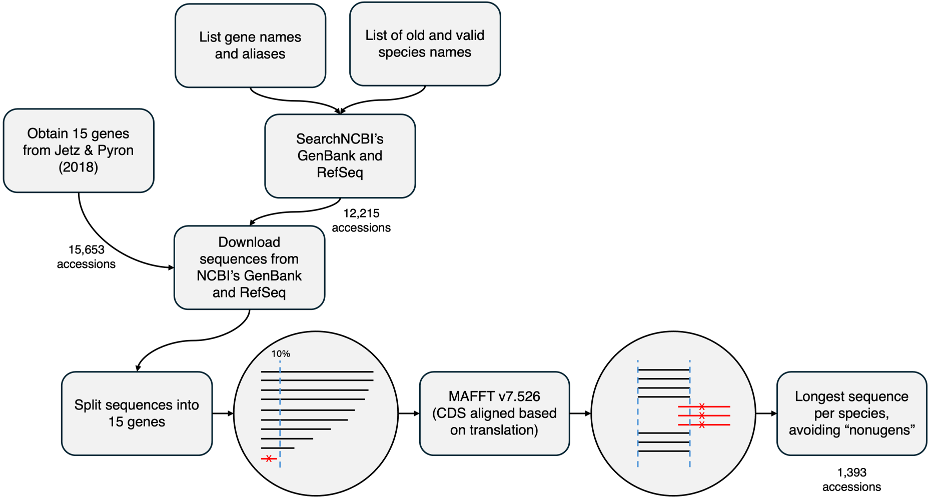


Vera Candioti, Dias, and Haas (*in press*)

They revisited Haas (2003) matrix and optimize it into Jetz and Pyron (2018) topology, proposing some synapomorphies different from those in Haas (2003).



Molecular data collection



List gene names and aliases

List of old and valid species names

Obtain 15 genes from Jetz & Pyron (2018)

SearchNCBI's GenBank and RefSeq

12,215 accessions

15,653 accessions

Download sequences from NCBI's GenBank and RefSeq

Split sequences into 15 genes

MAFFT v7.526 (CDS aligned based on translation)

Longest sequence per species, avoiding "nonugens"

1,393 accessions

Tree search, clade metrics, sensitivity analysis, and character categorization

Tree search:

- **Step 1:** PhyG, 10 independent replicates, equal weights, `search(hours:24, stop:10, instances:10, thompson:2, linear)` followed by `swap()`
- **Step 2:** TnT, 10 independent replicates, equal weights, `xmult= level 10 chklevel 5 rep 1000;`

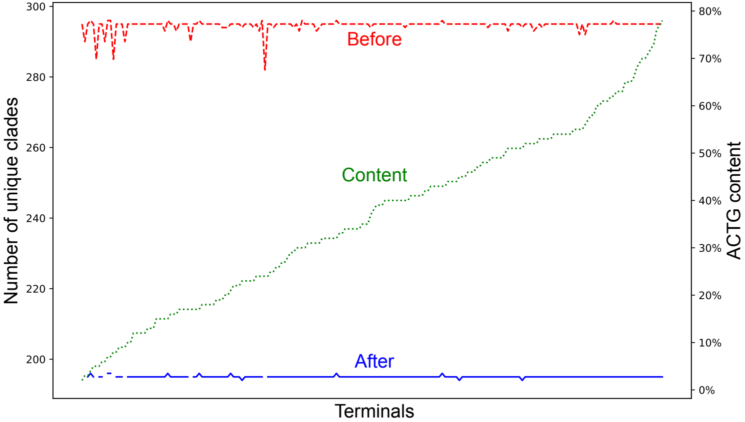
Jackknife and Goodman-Bremer calculations:

- **TnT:** command `resample jak replications 1000;`
- **TnT:** `Bremer.RUN` macro
- **TnT:** using `PhylogenomicSupport.run`

YBYRÁ:

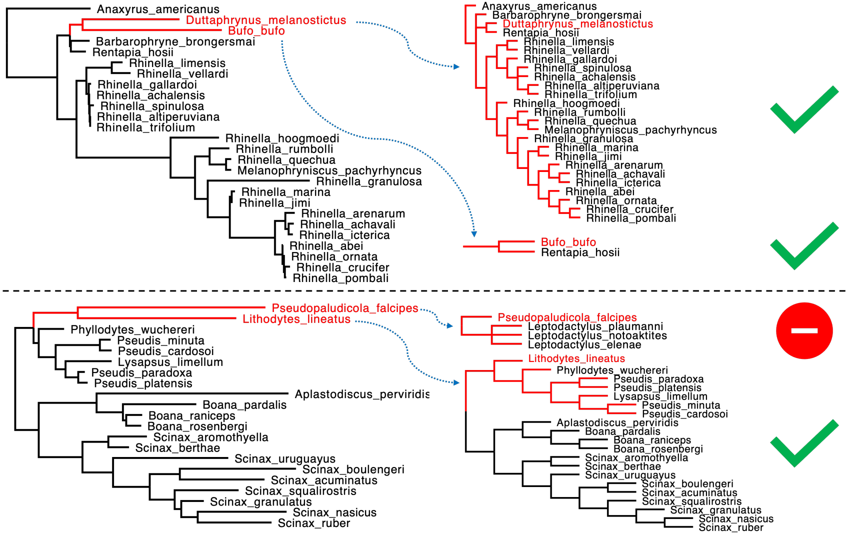
- Sensitivity analysis with `ybyra_sa.py`
- Wildcard search with `ybyra_wild.py`
- Character categorization with with `ybyra_apo.py`

Handling “nonugens,” misannotation, and chimaeric terminals



We found and removed 7 putative wildcards considering the polytomies they caused but also their ACGT content and metadata: *Cycloramphus lithomimeticus*, *Gastrotheca riobambae*, *Ikakogi ispacue*, *Leptodactylus latinasus*, *Leptodactylus pustulatus*, *Mannophryne herminae*, and *Peltophryne peltocephala*.

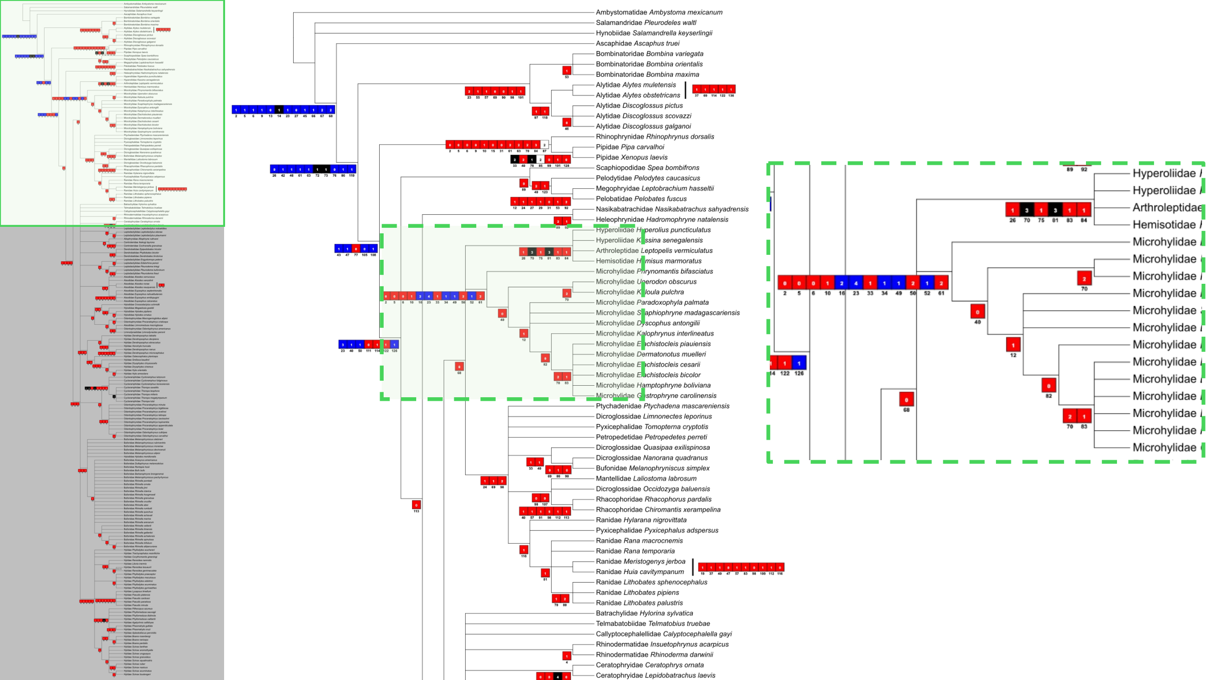
Long branch attraction



Resulting matrix and graphs

- 195 terminals
  - 195 species
  - 104 genera
  - 42 families
- 14,119 characters
  - 137 morphological
  - 13,982 molecular
    - 15 different molecular markers

- 1 PhyG graph and 2 most parsimonious trees from TnT
  - 71,365 steps
  - 1 polytomy caused by conflicting positions of *Rhinella achalensis* and *Rhinella spinulosa*


Ambystomatidae Ambystoma mexicanum
Salamandridae Pleurodeles waltl
Hynobiidae Salamandrella keyserlingii
Ascaphidae Ascaphus truei
Bombinatoridae Bombina variegata
Bombinatoridae Bombina orientalis
Bombinatoridae Bombina maxima
Alytidae Alytes muletensis
Alytidae Alytes obstetricans
Alytidae Discoglossus pictus
Alytidae Discoglossus scovazzi
Alytidae Discoglossus galganoi
Rhinophrynidae Rhinophrynus dorsalis
Pipidae Pipa carvalhoi
Pipidae Xenopus laevis
Scaphiopodidae Spea bombifrons
Pelodytidae Pelodytes caucasicus
Megophryidae Leptobrachium hasseltii
Pelobatidae Pelobates fuscus
Nasikabatrachidae Nasikabatrachus sahyadrensis
Heleophrynidae Hadromophryne natalensis
Hyperoliidae Hyperolius puncticulatus
Hyperoliidae Kassina senegalensis
Arthroleptidae Leptopelis vermiculatus
Hemisotidae Hemisus marmoratus
Microhylidae Phrynomantis bifasciatus
Microhylidae Uperodon obscurus
Microhylidae Kaloula pulchra
Microhylidae Paradoxophyla palmata
Microhylidae Scaphiophryne madagascariensis
Microhylidae Dyscophus antongilii
Microhylidae Kalophrynus interlineatus
Microhylidae Elachistocleis piauiensis
Microhylidae Dermatonotus muelleri
Microhylidae Elachistocleis cesarii
Microhylidae Elachistocleis bicolor
Microhylidae Hamptophryne boliviana
Microhylidae Gastrophryne carolinensis
Ptychadenidae Ptychadena mascareniensis
Dicroglossidae Limnonectes leporinus
Pyxicephalidae Tomopterna cryptotis
Petropedetidae Petropedetes perreti
Dicroglossidae Quasipaa exilispinosa
Dicroglossidae Nanorana quadranus
Bufonidae Melanophryniscus simplex
Mantellidae Laliostoma labrosum
Dicroglossidae Occidozyga baluensis
Rhacophoridae Rhacophorus pardalis
Rhacophoridae Chiromantis xerampelina
Ranidae Hylarana nigrovittata
Pyxicephalidae Pyxicephalus adspersus
Ranidae Rana macrocnemis
Ranidae Rana temporaria
Ranidae Meristogenys jerboa
Ranidae Huia cavitympanum
Ranidae Lithobates sphenocephalus
Ranidae Lithobates pipiens
Ranidae Lithobates palustris
Batrachylidae Hylorina sylvatica
Telmabatobiidae Telmatobius truebae
Calyptocephalellidae Calyptocephalella gayi
Rhinodermatidae Insuetophrynus acarpicus
Rhinodermatidae Rhinoderma darwinii
Ceratophryidae Ceratophrys ornata
Ceratophryidae Lepidobatrachus laevis
Hyperoliidae
Hyperoliidae
Arthroleptidae
Hemisotidae
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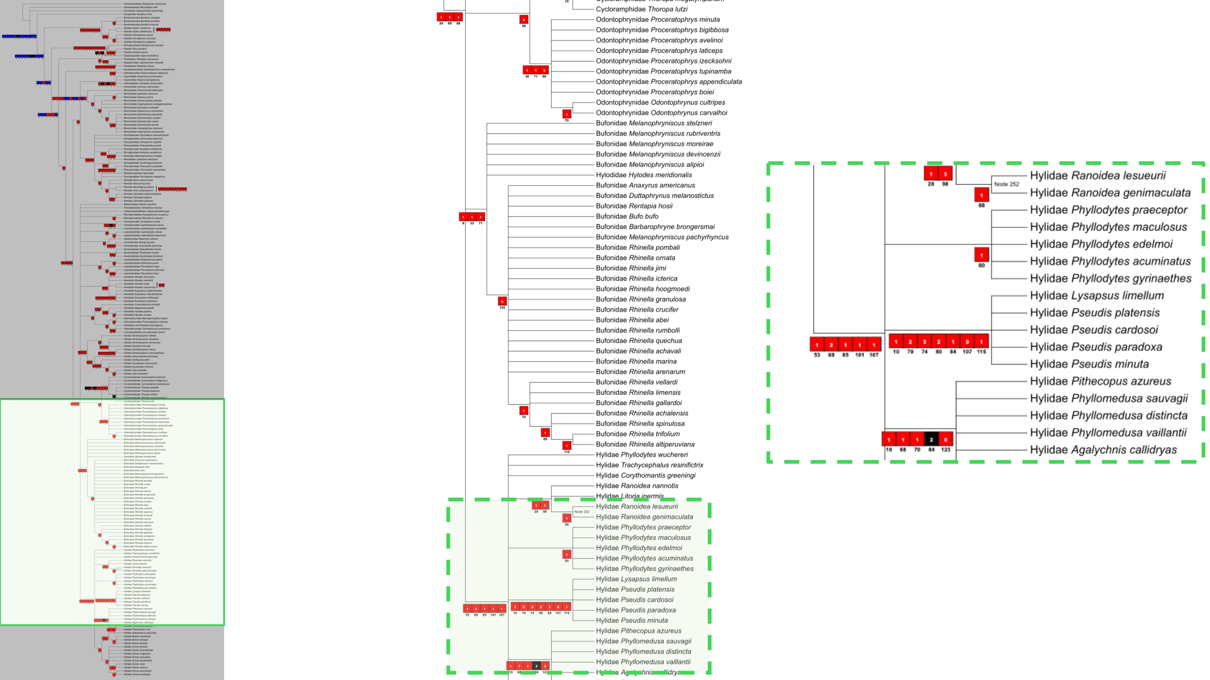
Leptodactylidae Leptodactylus notoaktites
Leptodactylidae Leptodactylus elenae
Leptodactylidae Leptodactylus plaumanni
Allophrynidae Allophryne ruthveni
Centrolenidae Ikakogi tayrona
Centrolenidae Cochranella granulosa
Dendrobatidae Epipedobates tricolor
Dendrobatidae Phyllobates bicolor
Dendrobatidae Dendrobates tinctorius
Leptodactylidae Engystomops petersi
Leptodactylidae Edalorhina perezi
Leptodactylidae Pleurodema kriegi
Leptodactylidae Pleurodema bufoninum
Leptodactylidae Pleurodema thaul
Alsodidae Alsodes verrucosus
Alsodidae Alsodes vanzolinii
Alsodidae Alsodes norae
Alsodidae Alsodes neuquensis
Alsodidae Eupsophus septentrionalis
Alsodidae Eupsophus nahuelbutensis
Alsodidae Eupsophus emiliopugini
Alsodidae Eupsophus calcaratus
Hylodidae Crossodactylus schmidti
Hylodidae Megaelosia goeldii
Hylodidae Hylodes pipilans
Hylodidae Hylodes ornatus
Odontophrynidae Macrogenioglottus alipioi
Odontophrynidae Proceratophrys cristiceps
Alsodidae Limnomedusa macroglossa
Odontophrynidae Odontophrynus americanus
Limnodynastidae Limnodynastes peronii
Hylidae Dendropsophus labialis
Hylidae Dendropsophus decipiens
Hylidae Dendropsophus ebraccatus
Hylidae Xenohyla truncata
Hylidae Dendropsophus nanus
Hylidae Dendropsophus microcephalus
Hylidae Osteocephalus planiceps
Hylidae Smilisca baudinii
Hylidae Dryophytes chrysoscelis
Hylidae Dryophytes cinereus
Hylidae Hyla orientalis
Hylidae Hyla annectans
Cycloramphidae Cycloramphus lutzorum
Cycloramphidae Cycloramphus fuliginosus
Cycloramphidae Cycloramphus boraceiensis
Cycloramphidae Thoropa saxatilis
Cycloramphidae Thoropa taophora
Cycloramphidae Thoropa miliaris
Cycloramphidae Thoropa megatympanum


Cycloramphidae Thoropa lutzi
Odontophrynidae Proceratophrys minuta
Odontophrynidae Proceratophrys bigibbosa
Odontophrynidae Proceratophrys avelinoi
Odontophrynidae Proceratophrys laticeps
Odontophrynidae Proceratophrys izecksohni
Odontophrynidae Proceratophrys tupinamba
Odontophrynidae Proceratophrys appendiculata
Odontophrynidae Proceratophrys boiei
Odontophrynidae Odontophrynus cultripes
Odontophrynidae Odontophrynus carvalhoi
Bufonidae Melanophryniscus stelzneri
Bufonidae Melanophryniscus rubriventris
Bufonidae Melanophryniscus moreirae
Bufonidae Melanophryniscus devincenzii
Bufonidae Melanophryniscus alipioi
Hylodidae Hylodes meridionalis
Bufonidae Anaxyrus americanus
Bufonidae Duttaphrynus melanostictus
Bufonidae Rentapia hosii
Bufonidae Bufo bufo
Bufonidae Barbarophryne brongersmai
Bufonidae Melanophryniscus pachyrhyncus
Bufonidae Rhinella pombali
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Bufonidae Rhinella jimi
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Bufonidae Rhinella crucifer
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Hylidae Phyllodytes wuchereri
Hylidae Trachycephalus resinifictrix
Hylidae Corythomantis greeningi
Hylidae Ranoidea nannotis
Hylidae Litoria inermis
Hylidae Ranoidea lesueurii
Hylidae Ranoidea genimaculata
Hylidae Phyllodytes praeceptor
Hylidae Phyllodytes maculosus
Hylidae Phyllodytes edelmoi
Hylidae Phyllodytes acuminatus
Hylidae Phyllodytes gyrinaethes
Hylidae Lysapsus limellum
Hylidae Pseudis platensis
Hylidae Pseudis cardosoi
Hylidae Pseudis paradoxa
Hylidae Pseudis minuta
Hylidae Pithecopus azureus
Hylidae Phyllomedusa sauvagii
Hylidae Phyllomedusa distincta
Hylidae Phyllomedusa vaillantii
Hylidae Agalychnis callidryas
Node 252
1 1 1
24 69 88
0
28
1 1 2
30 71 80
1
70
1 1 1
8 33 71
0
133
1
74
1
69
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115
1 3
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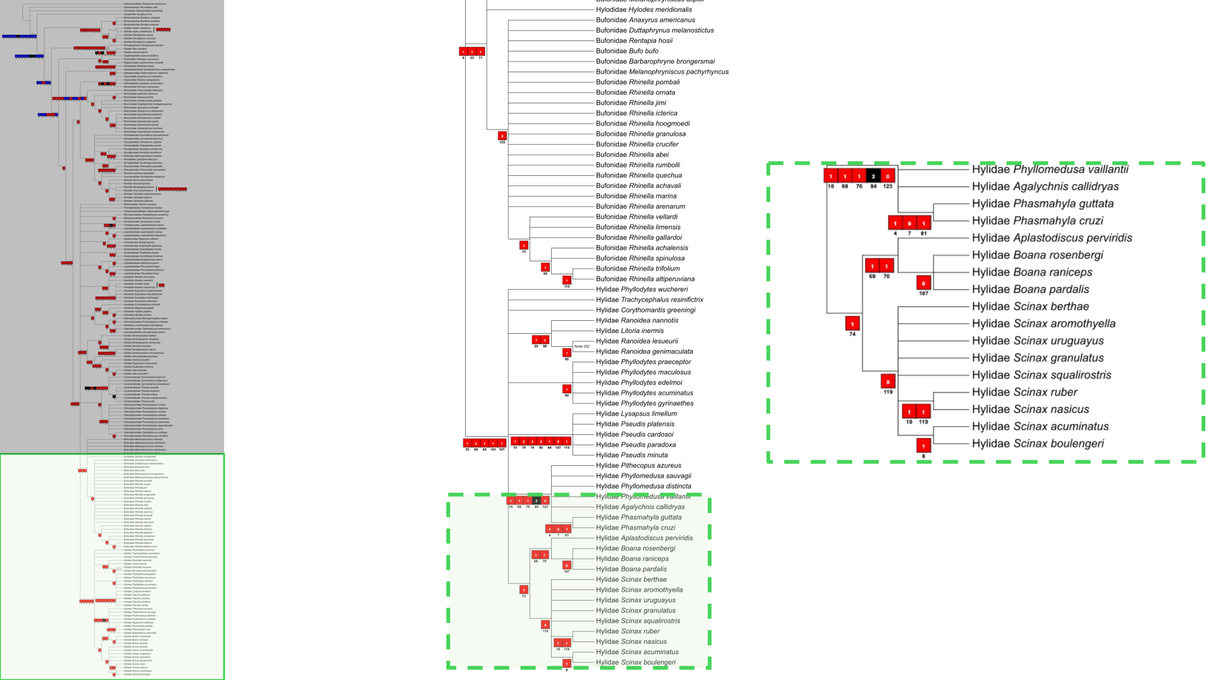
1 1 1 2 0
10 68 70 84 123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Hylidae Agalychnis callidryas
Hylidae Phasmahyla guttata
Hylidae Phasmahyla cruzi
Hylidae Aplastodiscus perviridis
Hylidae Boana rosenbergi
Hylidae Boana raniceps
Hylidae Boana pardalis
Hylidae Scinax berthae
Hylidae Scinax aromothyella
Hylidae Scinax uruguayus
Hylidae Scinax granulatus
Hylidae Scinax squalirostris
Hylidae Scinax ruber
Hylidae Scinax nasicus
Hylidae Scinax acuminatus
Hylidae Scinax boulengeri

Comparing matrices

Morphology:

- 137 characters
- 2 uninformative (1.46%)
- 979 transformations
- $26,716$ character states
- $5,601$ ambiguous states (20.96%)

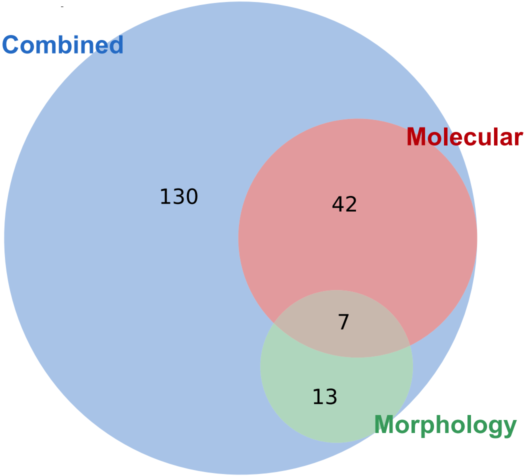
Molecules:

- $13,982$ characters
- $7,899$ informative (56.49%)
- $70,387$ transformations
- $2,726,491$ character states
- $1,775,411$ ambiguous states (65.12%)

Internal nodes with diagnostic synapomorphies:

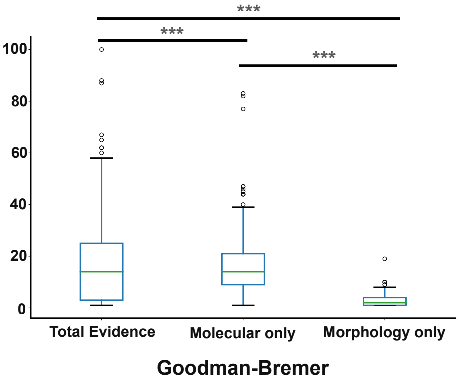
- Morphology only: 3
- Molecules only: 100
- Both: 84

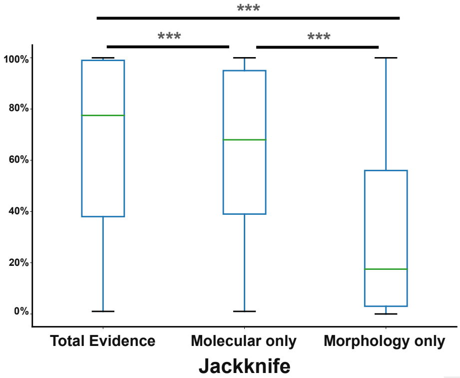
Combining morphological and molecular characters resulted in a topology that is mostly not recovered with molecular or morphological data alone.



The Venn diagram on the left shows the number of clades in the total evidence analysis and the amount of those clades in molecular- or morphology-only trees.

Goodman-Bremer and Jackknife values





Concluding remarks

- Our total evidence analysis of 15 genes plus 137 morphological characters yielded frog clades that would otherwise not be recoverable
- Our total evidence analysis resulted in more well-resolved trees with greater Jackknife or Goodman-Bremer values
- We raise concerns about nonugens, misannotations, and chimeric terminals on recent molecular phylogenies of frogs

Muito obrigado! **¡Muchas gracias!** **Thank You!**

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