World Congress of Herpetology Newsletter

June 2023, Volume 4, Issue 1

ISSN 2708-597X

www.worldcongressofherpetology.org

Making small animals big – the Tadpoles of Borneo Project

Written by **Alexander Haas[1] & Indraneil Das[2]**

[1] *Leibniz Institute for the Analysis of Biodiversity Change, Zoological Museum Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany. E-mail: a.haas@leibniz-lib.de*

[2] *Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia. E-mail: idas@unimas.my*

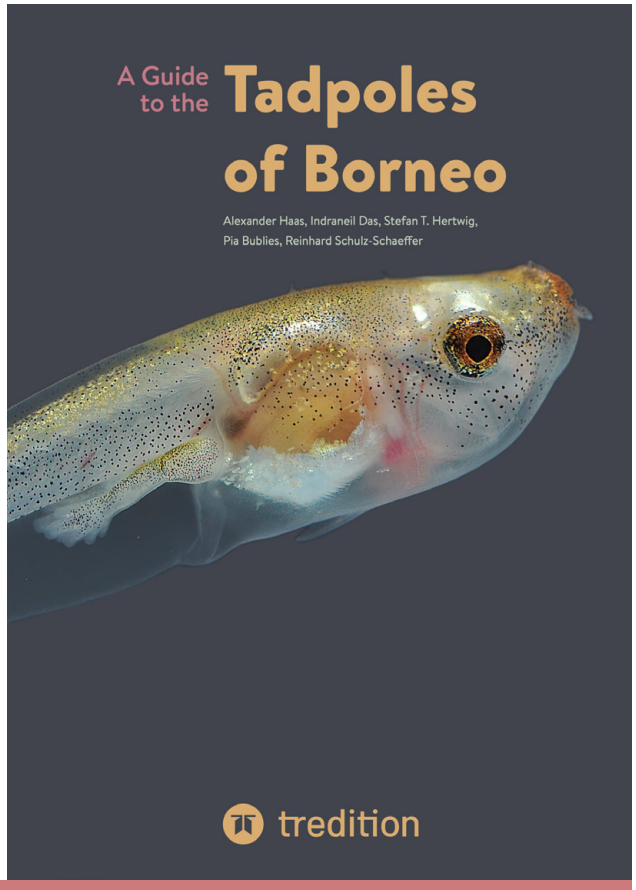
In the year 2004, two, then young, scientists with adventurous minds were anxiously sitting in a government office in Malaysia. They had submitted a research proposal to the authorities and were hoping to receive the pertinent permit papers. Whether a permit was about to be issued was unclear, as for some years before, the State had not been issuing research or export permits. They talked to the kind officer about the project and the officer clearly was struggling with the seemingly bizarre idea to study the tadpoles of Borneo. Why in the world would anyone make the effort to study such insignificant animals, he tried to reason. Of course, he was polite, and did not want to discourage, so he gave us this fatherly advice that it is a good thing to start with small animals and move to bigger ones. And how right he was! We left the office, grateful for the permit in hand, and started out on our big adventure- to catalogue the tadpoles of Borneo (and the frogs as well)!

Tadpoles are the larval life stages of most frog species. Tadpoles are part of a complex life-cycle that encompasses aquatic larvae, metamorphosis, and terrestrial adults. Larval stages play an important role in the biology of frogs and most other amphibians. In suitable water bodies, tadpoles can utilize several levels in the food pyramid, in extreme cases starting with bacteria and unicellular eucaryotes. Such food resources can be abundant and allow for quick increase in body mass of a tadpole. As many studies have shown, successful growth and development of the tadpoles have direct effects on performance and survival of the post-metamorphic frog. The fact that most frog species rely on this complex life-cycle only underlines its evolutionary success. It is a life-cycle that is not just a feature inherited from an distant ancestors but a state of being that is selected for because of survival benefits. All this, however, works to to the advantage of the species only if bodies of water with high primary food production are available. Some species have given up the free-swimming tadpole stage and bi-phasic life-cycle and undergo direct development in the egg. Such direct developers can be the predominant frog fauna in some areas where there are no oppor-

tunities for reproduction for bi-phasic species. On the island of Borneo, however, the vast majority of the currently recognized nearly 200 species follow the bi-phasic development and only a small fraction of species, namely some species in the genus *Philautus*, have been positively identified as direct developers (Hertwig *et al.* 2012).

We discussed the possibility of working on Bornean tadpoles as early as 2001, when Indraneil had invited Alexander to visit Borneo in order to do some first explorations of field sites. For Alexander it was the first trip to the region and Indraneil, who had been educated in India and Great Britain, had only arrived at his position at the Universiti of Malaysia Sarawak three years prior, with the establishment of the university's new Institute of Biodiversity and Environmental Conservation. Both of us were hyper-excited to explore the herpetofauna of Borneo. The first short reconnaissance trip together led us to the Kelabit Highlands and the Niah Caves. Soon the decision was made to combine our specific expertises and skills and submit a proposal to Volkswagen Foundation in 2003 with the objective to produce an inventory of the frogs and tadpoles of East Malaysia (Sarawak and Sabah).

The VW-Foundation grant application was successful the same year, and real work could begin after permits had been granted. The time for the start of the project was perfect for several reasons: For the inventory, we intended to document tadpoles in colour photographs. The previous work by Anstis (2002) and Chou & Lin (1997) had impressively shown that colour photo



Cover of the book *A Guide to the Tadpoles of Borneo*.

Fig. 1. The burrowing, eel-shaped tadpoles of *Leptobrachella mjobergi* live in the interstices of gravel beds of small to medium-sized streams. Their unusual lifestyle led to several interesting evolutionary changes in the musculo-skeletal anatomy of the spine and cranium (Haas *et al.* 2006).

documentation is so rich in information that we considered it mandatory in taxonomic descriptions of tadpoles. At the time, digital photography had just developed traction and the big camera manufacturers came out with first digital models that had sufficient resolution and image quality for serious applications in scientific research. Genetic barcoding is another essential tool in tadpole taxonomy, because assigning a tadpole to the correct species is not an easy task in a part of the world where new species had been discovered continuously and similar species may occur in the same area. At the time of the beginning of the project, genetic barcoding had been well-established and just become affordable enough even for larger numbers of samples on a routine basis. Genetic barcoding was and is the the ideal choice to establish reliability in tadpole identification; the wrongly assigned tadpoles published in the literature from the pre-genetic era attest to that. Last but not least, the excellent work of the past, particularly the valuable compilations in Inger (1985) set the foundation on which we could build on. His publications on the then known tadpoles of Borneo, although lacking photos of live tadpoles, helped tremendously to familiarize us with the larval diversity and their life habits. We consulted this work frequently in the beginning of our project because one question became imminently important early on:

Where to find the tadpoles?

This simple question turned out to be more difficult than expected, even with the ecological notes from Inger (1985). Bornean frog fauna is high in diversity but abundance is mostly quite low. The first two years of our project turned out to be frustrating and we started wondering if we would ever collect enough data to write a successful report to the sponsor at the end. Learning as we went along improved our skills and sharpened our perceptions. The more time we spent in the field, the more we were able to predict the microhabitats of species. We developed and built our own hand-net designs, photographic equipment arrays and capture techniques. We never used electro-fishing, although our predecessors had done so, because we wanted to reduce disturbance to the aquatic communities to a minimum and also wanted to learn, where in the aquatic

Fig. 2. *Polpedates macrotis* is a common frog in the forests of Borneo. It tolerates disturbed habitats and modified habitats to some extent. The larvae grow relatively large and possess an elaborate gill-filter. Typically these tadpoles are brown to olive but specimens of a population from the Matang range develop a colorful morph.

habitat the tadpoles were in concealment. Locating them under natural conditions seemed essential to us, and became a sort of hunting game at times. Soon, we realized that tadpole detection was far more successful at night. Experiences acquired in the field and constant improvements of the equipment and protocols soon paid. Spotting tadpoles in the field became routine, and a rich database, including thousands of images and a wealth of 16S DNA barcode sequences accumulated. From the early years, Stefan T. Hertwig (Natural History Museum Bern, Switzerland), then a student of Alexander, became our permanent collaborator who has since been contributing in all aspects of the project. We conducted field trips together and brought our research results to publication, often by giving specific projects to supervised students. Topics mostly centered around new species (such as, Hertwig *et al.* 2014, Das *et al*. 2014, Waser *et al.* 2016), phylogenetic relationships (for example, Hertwig *et al.* 2011, Hertwig *et al.* 2013, Flury *et al.* 2021, Etter *et al.* 2021), and tadpole taxonomy (for example, Haas *et al.* 2012, Oberhummer *et al*. 2014).

Fig. 3. Much of the ventral side of *gastromyzophorous* tadpoles is occupied by the oral disc and abdominal sucker. The photo shows *Meristogenys kinabaluensis*, a tadpole of up to 67 mm total lenth. The abdominal sucker attaches the body of the tadpole firmly to rock surfaces in fast, often turbulent water.

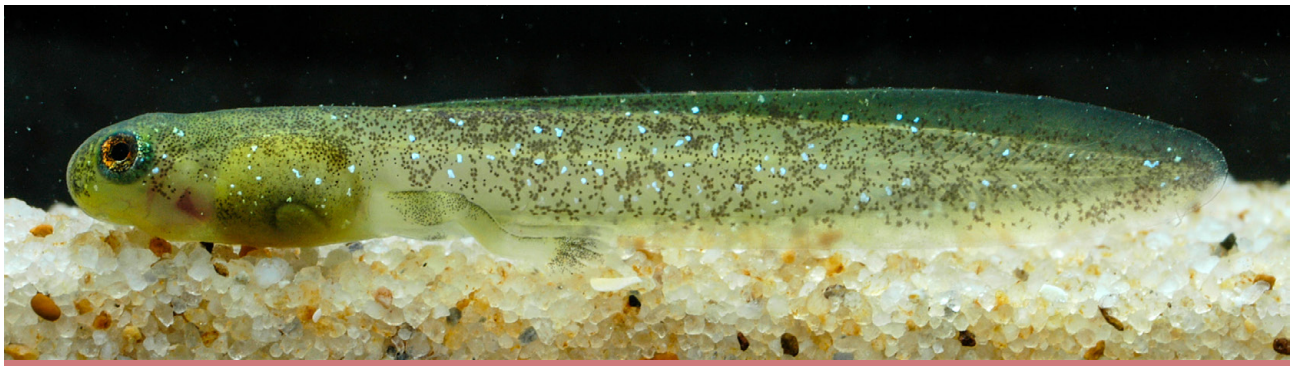


Fig. 4. Only one specimen of a larval *Philautus macroscelis* has been documented. The very short snout, lack of an oral disc and keratinized mouthparts, and presences of polygonal bluish-white iridocyte dots make it stand out from all other tadpoles on Borneo.

The application of barcoding allowed us to correct some misidentified tadpoles in the literature (Haas *et al*. 2009) and describe a number of tadpoles that had not been scientifically described before or needed an updated description (see citations above). Some cases were extraordinary indeed. On 19th September 2004, Indraneil had routinely swept a small stream on the upper reaches of Gunung Kinabalu with his net and was surprised to find a very strange-looking tadpole in his net. We stuck our heads together over the net and had a close look, but could not figure out the genus or even the family that this tadpole belonged to. Only later in the lab the case was solved by genetic barcoding. This discovery of a single specimen and highly unusual larval *Philautus macroscelis* resulted in taxonomic amendments and new hypotheses about the evolution of reproductive modes in Bornean *Philautus* (Hertwig *et al*. 2011). The modified *Philautus* larval morphology (short snout, reduced oral disk, reduced mouth opening) was later confirmed in the second, free-swimming larval form that we discovered and first described to science, *Philautus nepenthophilus* (Etter *et al.* 2021). Another memorable finding was the tadpole of *Leptobrachella baluensis*. On 13th August 2006, while down with fever, we encountered a small calling group of *L. baluensis* males along a forest path. The spot did not have water on the surface, however, water was running below the surface, along the gravel bed. Could there be tadpoles in the gravel? We figured that there was a possibility and began digging. And lo and behold, the hard work in this seemingly barren microhabitat finally surfaced a few of these remarkable fossorial tadpoles of that species. The burrowing tadpoles of *Leptobrachella* are undoubtedly a highlight in the tadpole fauna of Borneo, albeit difficult to find for the casual naturalist.

Fig. 5 *Microhyla nepenthicola* is one of the smallest frogs in the world. Males such as the one above typically measure 10.6-12.8 mm (females 17.9–18.8 mm). Eggs are deposited into ground-level pitchers of pitcher plants, particularly those of *Nepenthes ampullaria*. The tadpoles are very small, 9–11.3 mm in total length and do not feed (endotrophic).

The voucher specimens that we collected over the years gave us the opportunity to study the anatomy of *Leptobrachella mjobergi* in more detail (Haas *et al*. 2006). The species not only exhibits unusual arrangements of the cranial musculature, it also possesses a highly derived articulation of the first vertebra with the skull and larval vertebral column development that was later on shown to be found in other Megophryids as well (Handrigan *et al*. 2007). The highly derived head morphol-

ogy of gastromyzophorous tadpoles was highlighted in Gan *et al.* (2016) for *Huia cavitympanum* and *Meristogenys jerboa*, and that of the amazing carnivorous tadpole of *Occidozyga baluensis* in Haas *et al*. (2014).

In parallel to our team, other teams have contributed to the knowledge of anuran larvae on Borneo. First and foremost, Masafumi Matsui and, particularly his student Tomohiko Shimada published work together with multiple co-authors that included new and important tadpole data from Bornean species (among others, Shimada *et al.* 2015, Shimada & Matsui 2019). In sum, and building upon the excellent work by Inger (1985), knowledge on Bornean tadpoles grew substantially in the early 21st century. Yet, notable gaps in our knowledge remain. Surprisingly, for example, the tadpoles of some quite common species such as *Phrynoidis asper* and *Pulchrana baramica* have not been described scientifically and our team failed to discover tadpoles of these species in the field. Other groups defy the description of their larvae, because the taxonomy and phylogeny of the groups are unresolved (e.g., "kuhlii"-*Limnonectes*). Yet others reproduce secretively (*Kalophrynus*, *Pelophryne*, *Glyphoglossus*, *Gastrophrynoides*) or live as micro-endemics in remote localities (e.g., some *Ansonia* or *Pelophryne*). Locally performed breeding experiments could gather valuable information about the larval forms of such missing cases in the future.

We cannot help but mention a different topic that has lingered over biodiversity research for the past decades. We have been lucky enough to have the support of the Sarawakian and Sabahan authorities for almost 20 years. We would like to thank them for issuing permits and thus supporting our research. Field-collected voucher specimens are essential for our research and the field of taxonomy and systematics in general. Yet, in many tropical regions unauthorized activities by some individuals have negatively influenced the environment in which responsible biodiversity research can take place. The Malaysian territories on Borneo are no exception. Some scandals, cases of smuggling, and illegal fieldwork of various kinds (see the 2019 tarantula case, doi: 10.1126/science.aax1678) have increasingly sensitized State authorities and affected trust with those researchers who follow laws and regulations. Such negative effects by illegal activities are difficult to repair. Commonly, they

Fig. 6. Bornean *Philautus* have repeatedly been suspected to have direct development. Although this is true and has been confirmed for some species, others, such as *Philautus nepenthophilus* shown here, have free swimming larvae. *P. nepenthophilus* deposits eggs into pitchers several meters above the ground. The tadpoles have lost keratinized mouthparts and reduced the oral disc. They are endotrophic.

lead to more regulations and obstacles in connection with permitting processes. The Nagoya Protocol, originally designed, first, to standardize and facilitate procedures for non-commercial research and, second, to ensure shared benefits of the parties involved (Access and Benefit Sharing, ABS) has largely not yet brought the clarification and standardization of processes so many had expected (for example, Neumann *et al.* 2017). We hope that the implementation of the Nagoya Protocol mechanisms can be refined and adjusted further so that biodiversity research can continue to be performed under clear and transparent rules.

Public dissemination of research results is one of many ways of how to practice benefit sharing in the sense of ABS in bilateral collaborations. Early on in the project, we had the idea of publishing materials online in the form of a website, because we noticed the lack of an easily accessible resource on the Bornean frogs and their larvae, especially for southeast Asian students who might lack funds to buy the field guide available at that time (i.e., Inger & Stuebing 2005). We quickly created a small website. We wanted to keep it simple and present basic information and, most importantly, imagery of adults and larvae of as many of the recognized species as possible. At first, we started in a learning-by-doing phase with static pages, and after some years, we made the transition to a content management system driven website still operating today (Haas *et al.* 2022a). After going public, we were struck by the unexpected high number of clicks per day and soon realized that even with its rather basic and incomplete information the website was still welcomed by many users. We also learnt, however, that taxonomy and web technologies progress fast and that we underestimated the time needed for update and maintenance work for such a web resource, not to mention dealing with hacker attacks!

Finally in 2013, we decided to actually initiate the book project that we had already envisioned years before. We had not succeeded in collecting larval data for all Bornean species but we considered the data set solid enough for a book compilation. Another small grant from Volkswagen helped us to get started with the book. We were lucky to get additional collaborators and friends on board, Reinhard Schulz-Schaeffer, professor for scientific illustrations, and his former student, Pia Bublies, a freelance infographic professional. We opted for a print-on-demand publication model because we wanted to have complete freedom and control in realizing the project. We were ignorant, however, concerning the amount of work that such a self-publishing project would require. Composing the book in parallel with academic duties and demands proved to be challenging. In 2022, however, we were thrilled to present our book on Bornean tadpoles to the public (Haas *et al.* 2022b).

Working in the forest of Borneo has been an incredible experience and privilege for us. It seems that the scientific discoveries in Bornean forests will never end, and we sincerely hope that this unique place will be protected as a natural heritage for all mankind. We are grateful to all authorities, institutions, friends, field assistants, students, and many local helpers who have supported our work on Borneo for many years! For us, it was an exciting journey. And last but not least, we hope that the small tadpoles of Borneo will get the big stage they deserve!

References

Anstis, M. (2002) T*he tadpoles of South-eastern Australia*. New Holland Publishers (Australia), Sydney, Auckland, London, Cape Town.

Chou, W.-H. & Lin (1997) Tadpoles of Taiwan. *Special Publication National Museum Natural Science* 7, 1–98.

Das, I., Pui, Y.M., Hsu, W.W., Hertwig, S.T. & Haas, A. (2014) Red Hot Chili Pepper. a New *Calluella* Stoliczka, 1872 (Lissamphibia: Anura: Microhylidae) From Sarawak, East Malaysia (Borneo). *Zootaxa* 3785, 550–560.

Etter, L., Haas, A., Lee, C., Pui, Y.M., Das, I. & Hertwig, S.T. (2021) Out of the trap: A new phytothelm-breeding species of *Philautus* and an updated phylogeny of Bornean bush frogs (Anura: Rhacophoridae). *Journal of Zoological Systematics and Evolutionary Research* 59, 1064–1096.

Flury, J.M., Haas, A., Brown, R.M., Das, I., Pui, Y.M., Boon-Hee, K., Scheidt, U., Iskandar, D.T., Jankowski, A. & Hertwig, S.T. (2021) Unexpectedly high levels of lineage diversity in Sundaland puddle frogs (Dicroglossidae: *Occidozyga* Kuhl and van Hasselt, 1822). *Molecular Phylogenetics and Evolution* 163, 107210 (Online).

Gan, L.L., Hertwig, S.T., Das, I. & Haas, A. (2016) The anatomy and structural connectivity of the abdominal sucker in the tadpoles of *Huia cavitympanum*, with comparisons to *Meristogenys jerboa* (Lissamphibia: Anura: Ranidae). *Journal of Zoological Systematics and Evolutionary Research* 54, 46–59.

Haas, A., Das, I. & Hertwig, S.T. (2022a) Frogs of Borneo — The frogs of East Malaysia and their larval forms. Available from: http://www.frogsofborneo.org (2022)

Haas, A., Das, I., Hertwig, S., Schulz-Schaeffer, R. & Bublies, P. (2022b) *A Guide to the Tadpoles of Borneo*. Tredition, Hamburg, 279 pp. (shop.tredition.com)

Haas, A., Hertwig, S. & Das, I. (2006) Extreme tadpoles: The morphology of the fossorial megophryid larva, *Leptobrachella mjobergi*. *Zoology* 109, 26–42.

Haas, A., Hertwig, S.T., Krings, W., Braskamp, E., Dehling, J.M., Pui, Y.M., Jankowski, A., Schweizer, M. & Das, I. (2012) Description of three *Rhacophorus* tadpoles (Lissamphibia: Anura: Rhacophoridae) from Sarawak, Malaysia (Borneo). *Zootaxa* 3328, 1–19.

Haas, A., Pohlmeyer, J., Mcleod, D.S., Kleinteich, T., Hertwig, S.T., Das, I. & Buchholz, D.R. (2014) Extreme tadpoles II: the highly derived larval anatomy of *Occidozyga baluensis* (Boulenger, 1896), an obligate carnivorous tadpole. *Zoomorphology* 133, 321–342.

Haas, A., Wolter, J., Hertwig, S.T. & Das, I. (2009) Larval morphologies of three species of stream toads, genus *Ansonia* (Amphibia: Bufonidae) from East Malaysia (Borneo), with a key to known Bornean Ansonia tadpoles. *Zootaxa* 2302, 1–18.

Handrigan, G.R., Haas, A. & Wassersug, R.J. (2007) Bony-tailed tadpoles: the development of supernumerary caudal vertebrae in larval megophryids (Anura). *Evolution and Development* 9, 190–202.

Hertwig, S.T., Das, I., Schweizer, M., Brown, R. & Haas, A. (2011) Phylogenetic relationships of the *Rhacophorus everetti*-group and implications for the evolution of reproductive modes in *Philautus* (Amphibia: Anura: Rhacophoridae). *Zoologica Scripta* 41, 29–46.

Hertwig, S.T., Lilje, K.E., Pui, Y.M., Haas, A. & Das, I. (2012) Molecular evidence for direct development in the rhacophorid frog, *Philautus acutus* (Rhacophoridae, Anura) from Borneo. *The Raffles Bulletin of Zoology* 60, 559–567.

Hertwig, S.T., Pui, Y.M., Haas, A. & Das, I. (2014) Dressed in black: A new *Ansonia* Stoliczka, 1870 (Lissamphibia: Anura: Bufonidae) from Gunung Murud, Sarawak, East Malaysia (Borneo). *Zootaxa* 3814, 419.

Hertwig, S.T., Schweizer, M., Das, I. & Haas, A. (2013) Diversification in a biodiversity hotspot – The evolution of Southeastern Asian rhacophorid tree frogs on Borneo (Amphibia: Anura: Rhacophoridae). *Molecular Phylogenetics and Evolution* 68, 567–581.

Inger, R.F. (1985) Tadpoles of the forested regions of Borneo. *Fieldiana Zoology new series* 26, 1–89.

Inger, R.F. & Stuebing, R.B. (2005) *A Field Guide to the Frogs of Borneo*. Second Edition. Natural History Publications (Borneo).

Neumann, D., Borisenko, A.V., Coddington, J.A., Häuser, C.L., Butler, C.R., Casino, A., Vogel, J.C., Haszprunar, G. & Giere, P. (2017) Global biodiversity research tied up by juridical interpretations of access and benefit sharing. *Organisms Diversity & Evolution* 20, 1–12.

Oberhummer, E., Barten, C., Schweizer, M., Das, I., Haas, A. & Hertwig, S.T. (2014) Description of the tadpoles of three rare species of megophryid frogs (Amphibia: Anura: Megophryidae) from Gunung Mulu, Sarawak, Malaysia. *Zootaxa* 3835, 59–79.

Shimada, T. & Matsui, M. (2019) Re-examination of larval assignment of *Meristogenys poecilus* in Sarawak, Borneo, with a diagnostic table of *Meristogenys* larvae. 38, 23–31.

Shimada, T., Matsui, M., Nishikawa, K. & Eto, K. (2015) A new species of *Meristogenys* (Anura: Ranidae) from Sarawak, Borneo. *Zoological Science* 32, 474–484.

Waser, L.E., Schweizer, M., Haas, A., Das, I., Jankowski, A., Pui, Y.M. & Hertwig, S.T. (2016) From a lost world: an integrative phylogenetic analysis of *Ansonia* Stoliczka, 1870 (Lissamphibia: Anura: Bufonidae), with the description of a new species. *Organisms Diversity & Evolution* 17, 287–303. https://doi.org/10.1007/s13127-016-0294-2