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The Hemiphractid Frogs

Phylogeny, Embryology, Life History, and Cytogenetics

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Adult female (above) and male (below) of the marsupial frog *Gastrotheca walkeri*. These frogs were found approximately 15 m above the ground on a branch in the canopy at the immediate vicinity of the biological station 'Rancho Grande' in the Henri Pittier National Park in northern Venezuela (Aragua State). The field station (see fig. 167) is located in a cloud forest at an elevation of 1,100 m on the southern slopes of the Cordillera de la Costa (10°21′10′′N/67°41′00′′W). Here, a temporary cytogenetic laboratory was established in the years 1987, 1989, 1992, 1997 and 1998 by Michael Schmid, Claus Steinlein and Wolfgang Feichtinger. Together with Venezuelan colleagues, a large number of amphibian, reptilian and small mammalian species were collected in many localities in Venezuela, cytogenetically analyzed in this field station, and numerous fascinating karyotypes were discovered for the first time. Photograph by Michael Schmid.

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The Hemiphractid Frogs

Phylogeny, Embryology, Life History, and Cytogenetics

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Foreword

In 2010, Michael Schmid, James P. Bogart and S. Blair Hedges organized the monumental monograph *The Chromosomes of Terraranan Frogs*, containing detailed descriptions and illustrations of the karyotypes and genomes of 227 species of this major clade of anurans characterized by direct-development (one species is viviparous). It stands as the most complete cytogenetic study of a vertebrate group ever performed. Now, a mere two years later, Schmid and colleagues have produced the present volume, a work similarly rich in detail and coverage for the Neotropical frog family Hemiphractidae, a group in which the fertilized eggs are carried on the back, or in many others in a dorsal pouch, until hatching as a tadpole or small froglet. Study of the subject organisms and their karyotypes for the current monograph involved a dedicated commitment to collection and analysis carried out over a period of 43 years and included 16 expeditions to the American tropics (Central and South America, and Trinidad and Tobago). Although the focus of this study is on the chromosomes, the reader will be richly rewarded by paying attention to the exhaustive introductory sections on phylogeny, classification, biogeography, and especially those on reproduction, oogenesis, spermatogenesis, and early embryogenesis. Other sections of note are on developmental biology, life history and population declines in hemiphractids.

The major proportion of this book is given over to detailed descriptions of the karyotypes of 397 individuals representing 23 of the 100 species referred to the family and includes representatives of all genera except *Hemiphractus*. All karyotypes are illustrated by superb photographs selected from many hundreds taken. All modern techniques of preparation, including special staining, molecular probes, in situ hybridization, and genome measuring were the basis for data production. The extensive data sets are then used for the reconstruction of various karyophylogenies. These results are compared with cytogenetic published data for other amphibian taxa and other vertebrate groups and are supported by 1,300 literature citations. The final sections include discussion of structural and numerical repatterning of the family based on a background of general principles of chromosome evolution.

This monograph, like its predecessor *The Chromosomes of Terraranan Frogs,* raises and answers a wealth of fascinating questions on chromosome evolution, sex determination, meiosis, and cell biology while pointing out new questions to be considered. It will be especially of interest to recognized specialists in cytogenetics in general and specifically vertebrate cytogenetics for its elegant treatment of the material and issues of significance in the field. To the herpetologist, it provides the finely tuned treatment of the value of cytogenetic to phylogenetic reconstruction and the extraordinarily useful coverage of development, life history and biogeography. This opus thus stands as a *tour de force* in its own right!

Jay M. Savage
San Diego, Calif.
October 2012

Cytogenetic and
Genome Research

Preface

The first expedition to South America and chromosome analyses for the present monograph on Hemiphractidae were realized by James P. Bogart 43 years ago in 1969. In 1987, Michael Schmid, Claus Steinlein and Wolfgang Feichtinger started with their collecting trips and cytogenetic studies in Central- and South America as well as in the Caribbean region. During 1989 and 1990, Thomas Haaf was involved in the immunocytogenetic studies on hemiphractid cells and chromosomes. In 2010, S. Blair Hedges, Eugenia M. del Pino and William E. Duellman joined this project with their contributions on phylogeny, embryology and life history of hemiphractid frogs. In that same year, Indrajit Nanda initiated experiments to locate sex-determining genes in the karyotypes of these frogs.

The time required for writing the text of the manuscript, as well as for the preparation of the numerous karyotypes and other illustrations, was only two years. All correspondence and discussions on hemiphractid frogs between the authors in the USA, Canada, Ecuador, and Germany took place by electronic mail, courier messages containing photographic negatives, electronic transmission of huge files with illustrations, and numerous but always humorous 'late night' telephone calls. Not a single face-to-face meeting of the authors, prior to or during the elaboration of this monograph, was necessary to keep this project going and adequately finished. On the one hand this shows the efficiency of modern communication technology, on the other hand it is compelling evidence for the enduring enthusiasm of all authors in this project.

Although the karyotypes of hemiphractid species are rather conservative when compared to other groups of neotropical anurans, they raise and answer a wealth of fascinating questions on chromosome evolution, sex determination, meiosis, and cell biology. Furthermore, reproduction, embryology and life history of the Hemiphractidae belong to the most challenging aspects in herpetology and developmental biology. It is for this reason that these chapters have been included in the present monograph.

The present book comprises the chromosomes of only 23 of the 100 different recognized hemiphractid species, and one genus *(Hemiphractus)* could even not be included at all, although all efforts were made to obtain living specimens. One of the reasons is certainly the regrettable recent scarceness of many species in their former habitats, which is attributable, among other factors, to excessive human activities in the Neotropics. However, the wealth of cytogenetic data obtained from the 23 analyzed hemiphractid species provides a solid background to justify the publication of this book.

An adequate knowledge of phylogenesis is a necessary prerequisite for interpreting chromosome evolution in any group of eukaryotes. Most earlier comparative studies dealing with chromosome evolution in Amphibia were restricted by poor knowledge of their relationships. One of the few exceptions is an extensive analysis on terraranan chromosome evolution [Schmid et al., 2010] which used knowledge of their phylogeny down to the species group and species series level. In the present investigation, the evolution of hemiphractid karyotypes was also determined by mapping the karyotype characters on their molecular phylogeny to determine the most parsimonious arrangements.

In the present book, the results obtained for the analyzed hemiphractid frogs are always compared with cytogenetic data published for other amphibian taxa and other vertebrates or invertebrates. The deduced structural and numerical chromosome repatternings in hemiphractids are explained on the background of general principles of chromosome evolution. We have always aspired for a very high quality of histological sections, chromosome preparations and microphotographs, as well as for precise and explanatory graphics, schematic drawings and tables.

In spite of the detailed data presented here, we are aware that many questions concerning chromosome evolution in the Hemiphractidae remain unanswered. A careful cytogenetic examination of many additional species in the genera *Cryptobatrachus, Hemiphractus, Gastrotheca*, and *Stefania* is required in order to obtain a more complete picture of the fascinating chromosomes as well as of the karyophylogeny of these frogs. We also expect our interpretations on hemiphractid chromosome evolution will likely change in light of work including molecular cytogenetic techniques, especially those using in situ hybridizations with chromosome-specific DNA probes. Nevertheless, the information presented here provides a good basis and foundation for future research on these frogs.

The present book is the second volume in a larger series dedicated to amphibian cytogenetics launched by Karger Publishers. We hope it will be useful for providing detailed and archival information on hemiphractid karyotypes and genomes to recognized specialists in amphibian and vertebrate cytogenetics. We also expect that this monograph will encourage students to participate in future investigations on vertebrate cytogenetics.

Michael Schmid, Würzburg
James P. Bogart, Guelph, Ont.
S. Blair Hedges, University Park, Pa.
October 2012

Acknowledgements

The cytogenetic studies preceding the publication of the present monograph on hemiphractid frogs were carried out for 43 years (1969–2012), on 16 expeditions to Central- and South America, Trinidad and Tobago and in several laboratories. In a project of such magnitude, the authors asked for and received the support of many colleagues. The Editors of the monograph (Michael Schmid, James P. Bogart and S. Blair Hedges) acknowledge with great pleasure all the persons mentioned in the following and thank them for their invaluable help.

First of all, we need to thank all the colleagues, students and assistants for their essential logistic support in Central- and South America and on Trinidad and Tobago, for their immense and indefatigable engagement and assistance during all the expeditions, for their intrepidity in coping with all the adventures in the tropical environments, the rainforests, páramos, and altiplanos and/or for their skillful laboratory analyses on hemiphractid frogs. We thank Raymond Laurent (Argentina), Bertha Lutz†, Paulo E. Vanzolini, Henrique Rodrigues da Costa (Brazil), Richard Friedl, Martina Guttenbach, Christian Lomb, Anna Rohrbacher, Sabine Tauscher, Roland Weimer (Germany), Hinrich Kaiser (USA), José de Aguar, Mariana Albornoz, Marcos Alemán, Raúl Castro, David Diez, Alberto Fernández, Ernesto Fernández†, Marco Gaiani, Manuel Galea†, Jorge Gonzalez, Enrique La Marca, Jesús Manzanilla, Thomas Schmid†, Gregorio Ulloa and Richar Visbal (Venezuela).

Angela B. Marion and Molly Oberholtzer (Department of Biology, Pennsylvania State University, University Park, Pa., USA) kindly assisted with DNA sequence analyses of hemiphractid species and with graphic works, respectively. Matthias Greier (Department of Human Genetics, University of Würzburg, Germany) performed the fluorescence in situ hybridization experiments with several sex-specific DNA probes on *Gastrotheca riobambae* chromosomes.

We are very grateful to the following colleagues who have generously helped in obtaining living specimens or fixed tissues of hemiphractid species for our cytogenetic studies in Brazil (Carlos Gonzaga de Almeida), Ecuador and Peru (Norbert Juraske) and Venezuela (Richar Visbal). Their collecting trips were partially financed by private funds.

On our expeditions in South America and Trinidad and Tobago, numerous people helped to make the trips successful events. Their kindness, hospitality and appreciation of our projects on neotropical amphibians remain unforgotten. Personnel of the Departamento de Zoología Agrícola, University of Venezuela (Maracay) provided essential logistical support during five long stays at the biological field station 'Rancho Grande' in the Henri Pittier National Park.

We very much appreciate all the efforts of Gitta Hesse (Department of Human Genetics, University of Würzburg, Germany) for having produced hundreds of high-quality photographic prints of chromosomes and cell nuclei of hemiphractid frogs in her darkroom. Because of her tireless efforts and skill, the illustrations in the present monograph again are of magnificent quality. In the same Department, Ilse Neumann† very efficiently and patiently managed the administrative work that Michael Schmid repeatedly, and perhaps intentionally, forgot. Many students in this Department have assisted in cell cultures, chromosome preparations and banding analyses, in situ hybridizations, measurements of genome sizes (DNA flow cytometry), and in evaluating cell nuclei parameters of hemiphractid frogs. They all contributed to a constantly vivid and inspiring working atmosphere.

Michael Schmid especially thanks the former head of the Department of Human Genetics (University of Würzburg), Holger Hoehn, for having supported repeated expeditions to the Neotropics and for his deep interest in basic and evolutionary cytogenetics. He acknowledges the very helpful discussions about the biology, chromosomes and genomes of Hemiphractidae with colleagues at the Biocenter of the University of Würzburg and in many other institutions. His parents Thea Schmid† and Gottfried Schmid† (Caracas, Venezuela) rendered possible most of the expeditions to Venezuela. He deeply appreciates the numerous efforts and time of Heidi Flatzek and Wolf Flatzek (Würzburg, Germany), who, in a particular difficult period, took burden from his shoulders, allowing him to continue with scientific projects and to participate in various expeditions.

James P. Bogart especially thanks his former PhD supervisor, W. Frank Blair† (University of Texas at Austin, USA),

for supporting his chromosome research in Texas and in South America even though much of that work was peripheral to his thesis. He also wishes to thank his wife, Jo Ellen, for her capable field assistance in Argentina and Brazil.

S. Blair Hedges especially thanks his PhD supervisor, Richard Highton (University of Maryland, USA), and postdoctoral supervisor, Linda Maxson (then at Pennsylvania State University, USA), for encouraging and supporting his work on tropical frogs. He also thanks the US National Science Foundation for support of his work (NSF DBI 0850013).

Eugenia M. del Pino acknowledges the help of her former PhD supervisor, Asa A. Humphries, Jr.† (Emory University, Atlanta, USA), Herbert C. Macgregor (University of Leicester, UK), Richard P. Elinson (Duquesne University, Pittsburgh, USA), other international collaborators, and current and former members of her laboratory (Pontificia Universidad Católica del Ecuador, Quito, Ecuador). In particular, she acknowledges the assistance of Margarita Campos, Ruth E. Ruiz, Ingrid Alarcón, Iván M. Moya, and Oscar D. Pérez for the microphotographs of *Gastrotheca riobambae* oocytes and embryos. Transmission electron microscopy of *G. riobambae* oocytes was done with the support of Michael F. Trendelenburg and Ulrich Scheer (German Cancer Research Center, Heidelberg, Germany) and Joseph G. Gall (Department of Embryology, Carnegie Institution of Washington, USA). The study of interchromatin granule clusters was done with the help of Joseph G. Gall. The study of LAP2 isoform expression in frogs and fish received the support of Georg Krohne and Carmen Lang (University of Würzburg, Germany). Cortical granules of *G. riobambae* oocytes were investigated by Marcelo O. Cabada (Universidad Nacional de Rosario, Argentina) and Marta Gómez (Universidad Nacional de Tucumán, Argentina). The latter study received the support of a binational research grant from the Regional Latin American Program of the United Nations Development Program. The work conducted by Carmen Merizalde de Albuja† was possible thanks to a fellowship from the Latin American Regional Program of UNDP/UNESCO, and the support of Jorge E. Allende (Universidad de Chile, Santiago, Chile). The studies of monoclonal antibodies in *G. riobambae* oocytes by indirect immunofluorescence microscopy were supported by Peter Hausen† and Christine Dreyer (Max-Planck-Institute for Developmental Biology, Tübingen, Germany). William E. Duellman provided specimens, arranged loans from several museums and a visit to the Museum of Natural History of the University of Kansas (USA) which allowed analysis of the pouch, embryonic development and oogenesis of several species of hemiphractid frogs. He provided color photographs of hemiphractid frogs from the Museum collection. The photographs were taken by himself and by Thomas H. Fritts (Belen, New Mexico, USA).

William E. Duellman's first intensive fieldwork on marsupial frogs was performed in Peru with Thomas H. Fritts, Ismael Ceballos† and Oscar Ochoa in 1971. Later that year and in 1972 he made two trips to Ecuador for the expressed purpose of obtaining more material on marsupial frogs; during those trips he was accompanied at various times by Joseph T. Collins, Bruce MacBryde, John E. Simmons, and Linda Trueb. Much material was obtained during a 15-month trip through the length of the Andes in 1974–1975, during which time Dana Trueb Duellman, John E. Simmons and Linda Trueb worked diligently to enhance the collections. In 1977, David C. Cannatella joined him in the field in southern Peru, and in 1979 David C. Cannatella and Thomas J. Berger worked with him in northern and central Peru. Also in 1979, he ascended the slopes of Cerro Tamá on the Colombian-Venezuelan border; the success of that trip was due to the efforts of Amelia Díaz de Pascual and Jaime E. Péfaur. Fieldwork in Colombia in 1979 was successful thanks to the enthusiastic assistance of Fernando Castro. A collection of marsupial frogs was made in central Peru in 1983 with the aid of B. Anthony Luscombe and Patsy and Clarence J. McCoy†. Collections were made in Ecuador in 1984 with the determined assistance of Patricia A. Burrowes, David M. Hillis and John E. Simmons. Finally, Duellman's field studies on marsupial frogs were completed in Peru in 1989, where he was assisted by Fernando M. Cuadros, Michael E. Morrison, and John J. Wiens.

Karl-Heinz Jungfer (Gaildorf, Germany) provided color photographs of several living hemiphractid species and their progeny. The photographs of habitat loss in South America were generously provided by Rhett A. Butler (San Francisco, USA). Shirlei M. Recco-Pimentel (University of Campinas, São Paulo, Brazil) has helped in obtaining cytogenetic literature on Brazilian hemiphractids.

Our projects on hemiphractid frogs had considerable financial supports of national, academic and industrial foundations in the USA, Canada, Brazil, and Germany. These are listed in detail in table 7. In addition, the Department of Human Genetics of the University of Würzburg (Germany) generously contributed funds for the production of this monograph. We are grateful to the Governmental Authorities in Central- and South America and on Trinidad and Tobago for providing the necessary permits to collect amphibians within their territories and to bring them to our laboratories in Ecuador, Canada, Germany, and the USA.

Jacqueline Smith (Division of Genetics and Genomics, Roslin Institute, Roslin, Midlothian, UK), Associate Editor of the journal *Cytogenetic and Genome Research*, kindly processed the voluminous manuscript for publication.

The final draft of the manuscript was essentially improved thanks to the efforts of Martina Guttenbach (Managing Editor of *Cytogenetic and Genome Research*, Würzburg) and Karin Schmid who performed a thorough and fast copy editing. We are very thankful to the Publisher, Thomas Karger (Basel, Switzerland), for giving us the opportunity to publish the second large monograph in this series on amphibian biology, phylogeny and cytogenetics.

Remarks

Remarks on the Use of Scientific Names

Throughout the present monograph the scientific Latin names used for the species of Hemiphractidae and Terrarana follow the electronic database of Frost [2010] and the systematics and nomenclature of Crawford and Smith [2005], Heinicke et al. [2007, 2009] and Hedges et al. [2008a, b]. An exception are the species *Fritziana fissilis, F. goeldii* and *F. ohausi*, which were originally described as *Fritziana*, then transferred to *Flectonotus*, and recently retransferred to the genus *Fritziana* [Duellman et al., 2011b]. Common names of the hemiphractids and terraranans have been avoided.

The scientific names of all other amphibian species referred to in the text and tables are the same as were used in the original cited publications. Changes of scientific names of non-hemiphractid and non-terraranan amphibians are provided in Appendix I and are regularly updated in the electronic database of Frost [2010].

The scientific names used for all non-amphibian species referred to in the text are the same as in the original cited publications.

Remarks on the Illustrations, Tables and Appendices

The figures 1–159 are incorporated within the text of chapters 1.1–3.37. The legends to these figures are placed beneath or on the page before or after the corresponding illustrations. The color figures 160–168, showing the living terraranan species and their habitats, are placed on pages 332–340 and are referred to in the text. The tables 1–24 are incorporated within the text of chapters 1.1–3.37 and, when possible, are placed next to the pages where they are first referred. The Appendices I–III, which provide supplementary information, are found on pages 370–376.

Remarks on Previous Publications

The results of the cytogenetic studies on 14 out of the 23 hemiphractid species presented in this monograph were partially published in previous publications. These species are: *Cryptobatrachus boulengeri, Flectonotus pygmaeus, Fritziana fissilis, F. goeldii, F. ohausi, Gastrotheca cornuta, G. espeletia, G. gracilis, G. megacephala, G. ovifera, G. pseustes, G. riobambae, G. walkeri,* and *Stefania scalae* (table 8). In seven of these species *(F. pygmaeus, G. espeletia, G. megacephala, G. ovifera, G. pseustes, G. riobambae* and *G. walkeri)*, specific staining techniques and nucleic acid in situ hybridizations have been applied to the chromosomes. The chromosomes of the remaining seven species were only analyzed using conventional staining.

For the sake of completeness and for the convenience of the readers, theoretical cytogenetic background, modified figures, schematic drawings, and tables of our previous publications on Hemiphractidae are included in the present monograph. If necessary for a general understanding, the schematic drawings of other authors were redrawn in a modified form, and the source of the original drawing is included in the legend.

7 Traditional Classification and Phylogenies This phylogenetic tree is a reflection of the Linnaean classification of carnivores, however with the advancements in DNA and protein analysis, changes have been made in the traditional classification of organisms and their phylogeny. For example, birds are now classified as true reptiles. 8 Taxa A taxon is any group of species designated by name. Example taxa include: kingdoms, classes, etc. Every node should give rise to two lineages. If more than two linages are shown, it indicates an unresolved pattern of divergence or polytomy. 9 Sister Taxa Si