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Understanding cichlid evolution in the Neotropics

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Habilitation thesis:

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Part I.

Introduction

This thesis is based on a monograph paper titled “Diversity and evolution of the Middle American cichlid fishes (Teleostei: Cichlidae) with revised classification”. This paper reviews one geographical group of cichlid fishes, those of Middle America and is the largest, most complete and most multidimensional study of Middle American cichlids and of Neotropical cichlids ever published. The paper represents the culmination of research on this group of fishes and of my twenty years dedicated to this group. Description of the results this study has brought to our understanding of this particular group of fishes, of cichlids in general and of more general evolutionary and biogeographic questions will be given in Part III of the thesis. Here I would like to start by giving the reader a wider context to the origin of this study by presenting my publication record and my interests and motivations.

I am an explorer and naturalist at heart. This determines my interests in life, in biology, and in science. Within the biological fields I am an evolutionary biologist, vertebrate zoologist and biological systematist interested in exploration and discovery of biodiversity, its geographical distribution and its evolutionary, biogeographical and ecological origins. To make oneself viable as a field oriented scientist and explorer in today’s world of science forces one to specialize, if not analytically than at least taxonomically and geographically. For me this research specialization has been geographically narrowed on the Neotropics and taxonomically on freshwater fishes, in particular on cichlids. Professionally I however maintain a much wider spectrum of interests in biology and in other sciences which is reflected in my courses taught at FS USB, particularly in the interdisciplinary course of Biogeography.

All my projects (except for the earliest ones) strictly stem from my exploration and personal experience in the field. Opposite to the current trend I have always tended toward not specializing into one set of skills or interests (within the zoological boundaries) exploring various methodological approaches. My earliest projects before field exploration have been aquarium-oriented and focused on ethology and ontogeny. Slightly later I have added to this phylogenetic and evolutionary training at my home department (Zoology, FS USB) and the Universities of Stockholm and Copenhagen, molecular training first at the Institute of Entomology AS CR, later at the Institute of Animal Physiology and Genetics AS CR, the Swedish Museum of Natural History and the Museo Nacional de Ciencias Naturales (Madrid) and morphological training at Swedish Museum of Natural History. Only with my return at the end of my PhD studies became molecular studies feasible at my home department. My greatest scientific passion throughout has been biogeography, which is tightly connected with my exploratory nature. My prolonged stays in the wild parts of the Neotropics also deepened my interest and fascination with native peoples and their life and I have learned from them as much about the area-relevant nature as I have in all my formal schooling. Perhaps even more importantly I have learned some of the bushcraft skills that enable me to be comfortable in the rainforests and rivers of the tropics, the most magical places on this planet.

My set of skills enables me to use a multidimensional approach towards understanding the evolution of my model groups. I am thus a firm believer in a holistic approach as opposed to the presently dominant narrowly focused studies with a much lesser degree of synthesis. The monograph paper presented in this thesis is my first hint at the holistic approach of a synthesis that I strive to achieve for the Neotropical cichlids (and fishes) as a whole.

The majority of my publication record so far focuses on two main geographical areas (Middle America and southern tropical South America) of the Neotropics. Seven of my published studies have dealt principally with Middle American cichlids. An even larger number of my publications comes from my second main focal geographical area, that of

southern (sub)tropical South America centered in my case on the Argentinean province of Misiones, the last remaining still forested natural stronghold of the whole area, the once 1.5 million km² large Atlantic forest. Seventeen of my publications deal with freshwater fishes from this area, eight of them with cichlids and nine with other freshwater fishes. Work on this focal area started only with my postgraduate studies and thus lacks behind by about five years compared to my studies on Middle America. The visible imbalance between length of study and number of my results and final conclusions comes from our different knowledge of the faunas of Middle America compared to South America (much lesser in the later). This imbalance is actually even much greater than would appear from my publication record since my exploration since 2002 of the Neotropics has never been focused on the two before mentioned areas; these are just the areas that are much easier to come up with results in more than an anecdotal form.

My publication record is thus a narrow and biased reflection of my activities, endeavors and especially time dedication to my Neotropical projects and reflects only about ½ of my research activities. Among my nineteen field expeditions ten focused on the two focal areas from which most of my publication record comes while nine were dedicated to the exploration of the last wild large-scale part of the Neotropics, the Amazon. Additionally the planning and realization of the Amazonian expeditions were much longer than those to the surrounding areas reflecting the disproportion in time dedication and published results. The „problem“ (for me the beauty) with working in the Amazon and in providing circumscribed rather than anecdotal published information is its sheer size, ecological complexity, thankfully still very limited large-scale access and thus much lesser overall knowledge. The exploration of the Amazon is of a completely different nature, pace and short-term possible scope to the exploration of e.g. Middle America or southern tropical South America and this is the main reason that reflects itself in the very slow progress of both my publication record and our general understanding of this most biodiverse area of our planet. Amazonian cichlids feature predominantly in only three of my publications (cichlasomatine cichlids and *Crenicichla*), but the ratio within my publications will slowly shift towards more focus on the Amazon once the necessary level of completeness is reached in this mega-area to warrant a certain degree of completeness for publications (see below in Future directions). Another distinguishing factor is that my projects and efforts in the Amazon have never been directly funded and what has thus been done was from my own expenses and from money allocated from other projects and from elsewhere. While the Amazonian projects are as interesting and as important as those in my other areas the very long time needed to get the results is incompatible with standard research grant funding.

In my 19 expeditions so far (2002-2016) I have explored 13 Neotropical countries (6x Brazil, 5x Argentina, 3x Venezuela, 2x Mexico, 2x Peru, 2x Colombia, 2x Paraguay, Bolivia, Cuba, Costa Rica, Ecuador, Nicaragua, Panama) and have spend more than two years in the field. Hydrogeographically I have studied in all major river basins (but many smaller within the Amazon but still larger than any river in Europe with orders of magnitude higher diversity are still missing) with 9x Parana basin, 8x Amazon, 8x Middle America, 7x Uruguay basin, 4x Orinoco, 3x Paraguay basin, 2x Brazilian coast. Geomorphologically I have also collected in all major areas with 10x Brazilian shield, 8x Middle America, 7x Amazon, 5x Guiana shield, 5x Pantanal/Llanos/Savannas, 3x Andes. I have collected over 6000 cichlid specimens with DNA tissue samples including the majority of known Neotropical cichlid species (plus those that I have discovered and described as new to science and those that are known to me but require further analysis), with e.g. all but two cichlid species in Middle America, most species from multiple locations. Many areas however (fortunately!) still remain to be explored, virtually all of them in the Amazon (see above).

I have assembled through my own collections and from various other sources most likely the largest, but more importantly the most complete (taxonomically, biogeographically) DNA tissue collection of Neotropical cichlids in the World to study their diversity, phylogeny and evolution. Only one or two North American institutions have collections of approaching size, but these were assembled completely randomly and not specifically for the goals of a dedicated long-term study and thus feature enormous gaps both in species and area coverage and in species determination (a problematic issue of many resulting studies). Neotropical institutions have a tradition of collecting only their own country with some exceptions and have the same caveats as the North American collections. European collections have only localized representation of the Neotropical cichlids. Most Neotropical cichlid collections lack individual marking of specimens (samples) and their cross-linking with photographs of the specimens when alive (most lack photos of live specimens altogether) while coloration patterns are the single most important determination characters and most are lost upon museum preservation, making the material in many complex cases virtually useless.

My exploration of the freshwater fishes of the Neotropics features at two different levels in my publications: (1) one is the context of their diversity and diversification, which is reflected in my phylogenetic and historical biogeography studies; and (2) in the discovery of new species, new genera and new distribution records. Beyond these exploratory stages my publications further offer morphological, ecological or ontogenetic contexts. Ad 1. I have provided some of the first and more importantly the most complete phylogenies (both morphological and molecular) for various groups of Neotropical cichlids. Ad 2. I have discovered and described 14 new species (12 cichlid and 2 characid) and 11 new genera of Neotropical cichlids so far. I have also published a significant extension of distributional knowledge for many species of cichlid, characid, auchenipterid, loricariid, anostomid and pimeolodid fishes with 14 published first fish records for Argentina

Part II.

Overview of publication record

I have published 30 scientific publications, 21 with IF. I am the first author in 9 papers (2003-2016), the senior (last) author in 14 papers (2005-2016). Four of my papers are monographs / review papers (with 46 to 102 pages), 10 are standard length (~15 pages) research papers, 9 standard length (~15 pages) research systematic papers including descriptions of new species, 7 are short (2-5 pages) biogeographical notes (first distribution records), 1 is a short taxonomical note.

Nineteen of my publications deal with Neotropical cichlid fishes, 10 papers with other Neotropical (9) fish groups, 1 paper with a European mammal (*Spermophilus citellus*). Among the 28 Neotropical papers 7 have focus on Middle America and 21 on South America, of which 18 deal with southern tropical South America south of the Amazon basin.

The leading theme in my publications is biogeography (featured in 25 papers), 13 papers deal with systematics and descriptive morphology, 13 papers with various evolutionary aspects, 12 papers feature phylogenetic analysis, 5 papers morphoecology, 3 papers ontogeny, and one review paper includes all the above topics, is my largest publication and the main paper of this thesis.

I have pioneered studies of Neotropical cichlids in the Czech Republic (2003-present) and so far three of my PhD students (Musilová, Piálek, Dragová) have produced and co-authored significant publications. With Zuzana Musilová we have provided the first and so far only representative phylogenetic and biogeographic studies on the South American predominantly Amazonian cichlid tribe Cichlasomatini including the description of a new genus (papers 7,

11, 26). With Lubomír Piálek we have provided the first comprehensive phylogeny of the largest South American cichlid genus (*Crenicichla*) including a discovery of a new species flock within this genus and indications for sympatric speciation (paper 16) and the first comprehensive biogeography of the Middle American cichlids (paper 17) and with both Lubomír Piálek and Klára (Doubnerová) Dragová we have discovered and described several new species (papers 12-15, 18, 23, 25) and several new geographical distribution records (papers 10, 19, 21-22, 24, 27, 29-30). Both Lubomír Piálek and Klára Dragová have significantly contributed to the monograph paper presented in this thesis by working out the all important phylogenomic analysis that is the molecular analytical backbone of the study (paper 28). This monograph paper presented in this thesis is my first hint at the holistic approach of a synthesis that I strive to achieve for the whole of the Neotropical cichlids.

The following paragraphs delineate the necessary steps needed to understand the evolution of a given group (in my case of the Neotropical cichlids) as they have been applied in the monograph paper and in my other publications.

1) Diversity. The understanding of biodiversity comes through the understanding of systematics of a given group, which is the now completely overlooked and absolutely underfunded cornerstone of biodiversity research and biology in general. Thirteen of my papers are primarily systematic papers (papers 1, 3-4, 8, 11-15, 18, 23, 25, 28). Systematics and hence diversity is still incompletely known for Neotropical fishes in general (see BERTACO et al., 2016 for the latest) and for cichlids in particular as also my own research demonstrates; 12 new discovered and described species of cichlids (plus two non-cichlids) and 11 new genera, with many additional awaiting publication. The gain in knowledge in species systematics comes from revisionary studies and new species descriptions that are based both on available museum collections (5 new species in my case; papers 1 and 8) and own field work (9 new species in my case; papers 12-15, 18, 23, 25). The greatest of our new species discoveries and my greatest pride are two new species (paper 25) from a species flock also discovered by us in the Iguazu river (papers 16 and 30) above the Iguazu falls, which are the widest and in area largest and the most beautiful waterfalls in the World, a place visited every year by a million people; and we have been able to make such singular discoveries in this fantastic place. Currently we have in press the first review (paper 30) of the fish diversity of the Iguazu National Park, the only remaining natural area in the whole Iguazu river basin.

Many more new species are present in our data, especially for cichlids in the Amazon basin, many of which could superficially be labeled as cryptic species, which they however for the experienced eyes are not and which is confirmed by phylogenetic and recently through our phylogenomic analyses together with biogeography. The problem with the taxonomy of these mostly widely distributed taxa is that without the knowledge of their phylogeny and phylogeography it is very difficult to treat their geographical variation. In turn without having collected all (most) of them (within the assumed species groups) from their whole distribution area one cannot move forward with neither the taxonomical nor the evolutionary aspects of their diversity. We have now reached a point where most of the needed data is assembled and the phylogenies (phylogenomic analyses; see below) virtually completed. Based on our results at least thirty new cichlid species are thus already known in our datasets since many of the widespread taxa are actually polyphyletic or clearly phylogenetically structured by river basin or by geomorphological or ecological barriers and represent distinct morphological, phylogenetic and evolutionary species and more species still are to be expected from still unexplored areas of the Amazon. The clues for their search and discovery can only come from detailed knowledge of known distributions (however incomplete and artificially curtailed) combined with considerations gained from personal and published biogeographical, geomorphological and related ecological macro- and microscopical structuring of the Amazon basin.

2) Biogeography. Biogeography is the key to understanding biodiversity. Biodiversity derives directly from biogeography through isolation and/or ecological differentiation due to intrinsic factors and extrinsic factors stemming from geodiversity that determines drainage patterns, habitat ecology and vegetation formations. Biogeography due to its complexity is hence is my favorite subject which is reflected in it being the most common topic of my studies (present in 24 papers). The gains in knowledge about biogeographical distribution and biogeographical patterns come through exploration and new discoveries and through analytical results. Descriptive biogeography in the form of new distribution records is the topic of seven of my short papers (papers 10, 19, 21-22, 24, 27, 29-30) and biogeographical patterns of four of my papers (papers 5, 8, 14, 25). Since Neotropical institutions have a tradition of collecting only their own country with some exceptions, since there is in many cases great animosity between countries, and since the state of determination of species in museum collections is very often completely wrong the situation results into completely artificial distribution knowledge demarcated by artificial country borders in many fish taxa in many areas; hence the importance of cross-border faunal studies such as those of my faunal notes.

Analytical biogeography as the main topic is present in six of my papers (papers 7, 16, 17, 20, 26, 28). Comparative biogeography, in my case between cichlids and non-cichlids (and other animal groups) informs, strengthens and contrasts the specific cichlids patterns.

3) Ecology and morphology. Ecological and morphological characters are the main indicators of adaptation and diversification. Morphological characters and their analysis appears in my publications in the form of taxonomical descriptions and in the delineation (diagnosis) of taxa on one hand (see systematics papers in Diversity above), in phylogenetic analyses (see phylogeny below) and in the discovery of adaptive characters correlated with the environment and autecology of the taxa (papers 6, 8, 11, 25, 28). Two of my papers study in detail the evolution of cranial morphology in respect to prey capture (papers 6 and 28), the monograph paper (28) studies the complete evolution of morphology in ecological context in one clade of cichlids. Ecological characteristics of habitats of the studied taxa determine their adaptive trajectories and are one form of biogeographical determinants and are derived directly from the underlying geomorphology and geomorphological history of the given area. As such ecological factors play importantly in our biodiversity studies.

4) Phylogeny. Phylogeny and phylogenetic analysis is the cornerstone of evolutionary biology and its main analytical and interpretative framework. These days it has become purely molecular, while early on it was purely morphological. My research has gone through all the stages from morphological (dominating my undergraduate studies and also present in papers 4, 9, 11, 14-15, 23, 28) through various innovations of the molecular approaches, first solely mtDNA (papers 4-5), then the addition of nDNA, first as standard Sanger sequencing in concatenated multilocus approaches (papers 6-7, 9, 11-12, 15-17, 26, 28) and recently through NGS (paper 28 and all currently ongoing studies). NGS nDNA phylogenies are now completely dominating our research in combination with mtDNA for the maternally inherited signal.

5) Ontogeny. Understanding of mechanisms regulating expression of traits and generating divergence of characters is a key facet of evolutionary biology. Ontogenetic studies are virtually entirely of a laboratory nature, but when the mechanisms and indicators become understood even isolated records from the field and from collections can be incorporated. Ontogeny of characters feature in several of my studies (papers 2, 4, 9, 11, 28) and was predominantly focused on the very complex characters of coloration patterns. Two of the papers are monograph papers (papers 2 and 28), with paper two being entirely dedicated to the ontogenetic topic.

6) Speciation. Speciation takes two main forms, (1) allopatric speciation that is „easily“ studied by combining biogeography and phylogeny (papers 4-5, 7, 14, 16-17, 26, 28), and (2) sympatric speciation that is triggered ecologically without biogeographical contribution (papers 16, 25, 28). Speciation processes per se are the newest topic on our research plate, but this topic is also the one that offers some of the most interesting biologically general and widely applicable results and that thus promises and offers the greatest potential for attracting research money, students, scientific interest, cooperation and publicity as is already being demonstrated. One of our postdoc proposals has been financed, lost of feedback and interest is coming from conference meetings and we have started cooperation with a US based team thanks to these interesting topics and the genomic expertise that my former student and current postdoc Lubomír Piálek has developed.

Sympatric speciation and adaptive radiation in cichlids takes usually the form of species flocks (GREENWOOD, 1984; MAYR, 1984; VERHEYEN et al., 2003; SALZBURGER & MEYER, 2004) and we have discovered in the field and later confirmed through further research both ancient (paper 17, 28) and ongoing (paper 16, 25) species flocks. We are recently studying both types of species flocks (including some additional models except those in the above papers, e.g. the genus *Herichthys* in Middle America) using all the above approaches with NGS phylogenomics (and multivariate geometric morphometrics) as the central tool. In our model of ongoing sympatric speciation in the South American genus *Crenicichla* we are studying parallel evolution between two unrelated species flocks in two parallel river basins (the Uruguay and the Iguazu/Middle Parana; the latter species flock we have discovered in paper 16) as well as possible parallel speciation within the Uruguay species flock, where most species are phylogenomically polyphyletic (and some are paraphyletic) and appear to have evolved repeatedly in many major semi-isolated tributaries of the Uruguay river, in most of them with divergence of the ecomorphological forms (parallel species) in sympatry. The parallel evolutions and parallel speciations clearly demonstrate the strong force of ecological adaptation in speciation and in these particular cases also determinism of evolution with predictable outcomes. These speciation studies are also extremely interesting for the very general biological problem of what actually are species? These particular cases seem to support the notion that species are in many cases rather temporary units (existing as a cohesive unit only shortly after speciation), to a certain extent artificial and less „watertight“ biological concepts than populations, which are the true evolutionary units with the potential of developing new (apomorphic) traits in response to local evolutionary pressures (e.g. upon dispersal or even in situ due to ecological gradients etc.) and thus rendering the rest of the species „paraphyletic“ until eventually outcompeted and forced into extinction or reabsorbed into other entities. Further „borrowing“ or lateral transfer of already established genomic adaptations through hybridizations (something that we are testing now in our model groups to disentangle from true sympatric speciation) renders the speciation process even more complex and this mosaic type of speciation appears to be very common even outside adaptive radiations, most likely even in the evolution of our own species with foreign genomic regions in our genomes from Neanderthals (GREEN et al., 2010; PRÜFER et al., 2014), Denisovans (REICH et al., 2010; RASMUSSEN et al., 2011) and even older so far unidentified hominin species probably of the rank of the ergasters/erects (LACHANCE et al., 2012; some of this diverging genomic material could however also derive from differential survival of common ancestral polymorphisms; LOWERY et al., 2013). Our speciation studies thus have the potential to really see evolution and speciation in action and the so far least explored Amazonian speciations offer the prospects of studying this localized within-species (as well as „normal“ between-species) evolution and adaptation in another different context given the often very large distribution areas and sometimes quite heterogeneous ecological

settings and ecological gradients and clines in and along which nominal species are known to live.

7) Evolution. Evolutionary understanding is a synthesis from the above topics with emergent properties. Several of my papers study evolution of isolated topics (e.g. biogeography, morphology; see above) while paper 28 represents a complex evolutionary study of one clade of cichlids incorporating all the above topics and approaches. Studies of a similar scope to paper 28 are now being prepared for all groups of Neotropical cichlids in my lab. Paper 28 dealing with the Middle American cichlids took twenty years to assemble the necessary data. For the South American cichlid groups I have been assembling the data all the same time and it will take many more years to complete.

The building of the above research topics into a single cohesive evolutionary story is the main objective of the monograph paper the synopsis of which follows and which forms the core of this thesis. This paper is the first chapter in my efforts at providing a complete understanding of the evolution of the Neotropical cichlids set into the context of their continent, its biogeography, its other biodiversity and its evolutionary mechanisms.

Part III.

Understanding cichlid evolution in the Neotropics: Diversity and evolution of the Middle American cichlids

Řičan O, Piálek L, Dragová K, Novák J (2016) Diversity and evolution of the Middle American cichlid fishes (Teleostei: Cichlidae) with revised classification. *Vertebrate Zoology* 66: 1-102.

The study is the result of a team of three scientific generations. LP is a former student and current postdoc of OŘ, KD a current PhD student of OŘ and JN the former supervisor of OŘ. JN brought cichlids to my attention and provided me with undergraduate supervision and orientation into the biology of Middle American cichlids. LP and KD did most of the molecular lab work in this study and all of the newly generated genomics, which was pioneered in our lab by LP.

The study represents the largest, most complete and most multidimensional study of Middle American cichlids and of Neotropical cichlids ever published with a taxonomically complete and topologically robust molecular phylogeny based on which we have reviewed their diversity, genus-level systematics, biogeographical, ecological, morphological and life history evolution.

In order to ascertain the diversity of the group (at present with ~120 valid species plus three in the Greater Antilles) and its phylogeny we have used three nested taxon sampling analyses of the concatenated nDNA/mtDNA datasets and additionally to these analyses we present for the first time in this group of cichlids and for Neotropical cichlids a new Next Generation Sequencing-generated (NGS) nuclear phylogeny.

The NGS reduced genome representation method called double digest Restriction-site Associated DNA Sequencing (ddRADseq; PETERSON et al. 2012) was used to acquire a sufficient amount of nuclear markers for phylogenetic reconstruction. The resulting NGS ddRAD SNP matrices ranged in size from ~ 45,000 to 370,000 informative characters depending on the selected approach and parameter settings. The NGS ddRAD phylogeny is based on 240 samples representing 105 species of the Middle American cichlid clade plus 18 outgroup species and thus has a sampling covering virtually all species (including the enigmatic *Cichlasoma microlepis* DAHL, 1960) with multiple sequenced specimens from

different localities per species. Both assembling strategy and matrix size have very little effect on the tree topology; relevant values of branch supports slightly grow with the matrix size but in all analyses virtually all nodes have the maximum bootstrap support value of 1. A summary consensus topology based on both de-novo and reference-mapping analyses on the genome of *Oreochromis niloticus* has been used throughout the study as an analytical backbone to study the evolution of the group. The NGS phylogeny confirmed but significantly strengthened most previous phylogenetic results obtained from classical Sanger sequencing and concatenated dataset analyses, confirmed and even extended the cytonuclear discordances reported in our previous papers, resolved the previous soft polytomy of the amphiphine radiation, discovered a new clade of eastern Panamanian cichlids within the amphiphine radiation, supported a division of Middle American cichlids into three clades (with monophyletic astatheroines) and provided a firm resolution of the basal branching.

Our nDNA ddRAD topology reveals many cases of cytonuclear discordance at the species, genus and deeper levels including both introgressive hybridization and likely hybrid speciation stressing the importance to study the nuclear and mitochondrial phylogenetic signals independently (which has been done virtually only by our team in this group of cichlids) and not solely in concatenated analyses as is the norm in studies by other teams working on Neotropical cichlids. We have also found many instances of species misidentifications and/or contaminations, or simply the use of wrong sequences in many previously published studies by other teams. We have discovered these incorrect sequences by initial first-step analyses which analyzed all molecular markers separately by including all available sequences from GenBank and in the second step by comparisons of the sequences with the population-level sampling analysis based on specimens from multiple localities collected by us in the field.

We have reviewed the species level diversity of the Middle American cichlids using (1) the mtDNA *cytb* gene *population-level* analysis (with 903 terminals with 445 new *cytb* sequences representing all but one described and putative ingroup species of Middle American cichlids) with a backbone of the multilocus *genus-level* dataset and (2) the ddRAD analysis. The sequencing of the *cytb* gene from the new material and the ddRAD data were generated by LP and KD while OR collected all the 445 newly sequenced specimens that also include all specimens used for the ddRAD analysis in the field between 2002 and 2014.

Based on our results the Middle American heroine cichlids are made up of three main clades. The three clades (the herichthyines, the amphiphines, and the astatheroines) are however not each other sister groups since they are interspersed with South American (e.g. *Australoheros*, *Caquetaia*, *Mesoheros*) and Antillean (*Nandopsis*) genera and represent two separate colonization events of Middle America from South America, probably via the Antilles.

Our analysis of the diversity of Middle American cichlid clade supports the existence of 31 genera in Middle America (plus six in South America and one in the Greater Antilles) as separate evolutionary lineages occupying separate adaptive zones. We provide a complete review of the classification of Middle American cichlids (on 25 pages) with re-diagnoses of all genera and the description of nine new genera for species and species groups that have lacked a genus level name to this day or were associated with other unrelated genera. We have strived in the study for a balanced and stable solution to generic classification by following the proposed taxon naming criteria (TNCs) of VENCES *et al.* (2013) and have thus tried to eliminate both the objective and subjective reasons for the instability of classifications. The guidelines for classifications were ordered by VENCES *et al.* (2013) based on the order of importance. The *Primary* TNCs include: 1) Monophyly. Monophyly is the first, and the only strict taxon-naming criterion; 2) Clade Stability, i.e., the monophyly of a clade to be named as taxon should be as strongly supported as possible by various methods of tree inference, tests

of clade robustness, and different data sets; 3) Phenotypic Diagnosability, i.e., ranked supraspecific taxa should be those that are phenotypically most conspicuous (although in phenotypically cryptic groups of organisms it can be warranted to name taxa based on molecular differences alone); The *Secondary* TNCs include: 4) Time banding, i.e. evolutionary age as a criterion; 5) Biogeography; 6) Adaptive zone, i.e. a clade occupying a distinctive adaptive zone should be assigned to a ranked category and thus named as Linnaean taxon; and finally the *Accessory* TNCs include : 7) Manageable units (species-rich taxa can be advantageous if they are phenotypically homogeneous, phenotypically diverse are more manageable when partitioned into multiple genera); 8) Community consensus.

Diagnoses of genera are based on the possession of unique characters, character states or a unique apomorphic character state combination. We however also propose new genera for long-isolated and in DNA characters unique monotypic lineages that have ancestral morphological character combinations because such taxa would otherwise remain lingering in the taxonomical limbo because they are undiagnosable using morphological characters only.

We have analyzed all morphological characters previously used in describing the diversity and phylogeny of the Middle American Cichlidae. We have reconstructed the morphological ancestor of the Middle American cichlids by tracing the evolution of all morphological characters on the nDNA ddRAD phylogeny and this character mapping analysis was used for the formulation of the diagnoses of new genera and for revised diagnoses of existing genera. We have further contrasted morphological phylogenies derived from these morphological characters with the molecular phylogenies and have discovered that morphological characters in their majority do not reflect phylogenetic relationships but reflect ecological adaptations to similar environments and life styles.

We have thus discovered that a great majority of morphological characters are ecologically correlated and that they form only a limited number of functionally-determined combinations – i.e. ecomorphs. This has been confirmed both by (1) the contrasts between morphological phylogenies (which group these ecomorphs as clades) and the molecular phylogenies (which find them at the genus-level separately evolved) and (2) by PCA analyses of geometric morphometrics which recovers the same major ecomorphs as virtually separate PCA clusters. This is true for both the cranial as well as the postcranial characters. The cranial ecomorphs are determined by food choice, while the postcranial ecomorphs are determined by habitat characteristics. The morphological characters are not only correlated with each other and with ecology, but they also in the majority of cases show concerted evolution. We have found five major cranial ecomorphs (the ancestral generalists, predators-piscivores, detritivores-herbivores, periphyton scrapers and substratum sifters) but only two postcranial ecomorphs (the lotic and lentic ecomorphs, plus the undifferentiated ancestral character combination). The cranial and postcranial ecomorphs are not combined completely randomly and out of fifteen possible combinations have produced thirteen modular whole-body ecomorphs. Both the cranial and postcranial ecomorphs, and even their combinations, have evolved repeatedly in the Middle American cichlids in the same habitats in sympatry as well as in allopatry.

The evolutionary uniqueness of genera thus cannot be determined without the knowledge of phylogeny because of the character dependence with ecomorphs which has been the reason for the long struggle in classifying the Middle American cichlids into genera. In many cases molecularly identified unique evolutionary lineages (genera) have very few characters that enable independent diagnosis of parallel genera. Most often the main distinguishing characters are found in biogeography and in coloration patterns, in the latter especially in their ontogeny and in breeding coloration. However we have still found some indications that even the breeding dresses are under ecological and not only sexual selection. There is a strong correlation between the clear-water lotic ecomorph and a contrasting white-dominated

breeding coloration, while the turbid-water lentic ecomorph is characterized by a more obscure breeding coloration. This lentic-lotic breeding dress dichotomy exists irrespective of genus-level affinities at the species level.

The lentic-lotic dichotomy is most rampant within the terminal herichthyines (the genera in the *Theraps-Paraneotroplus* clade) where the lentic-lotic ecomorph dichotomy was responsible for great confusion in genus-level classification and very likely also for the unparalleled species diversity within this clade due to repeated evolution of the lotic and lentic ecomorphs. On the contrary we have not been able to find ecological correlates for the diversity found in the coloration pattern ontogenies and this character set thus remains the only one known to us that appears free of the ecological determinism dominant in the evolution on the Middle American cichlid fish diversity.

Life history traits evolution analysed and reviewed in the study includes coloration patterns, their ontogeny, breeding coloration, reproductive strategies and family type arrangements, with the r/K ecological strategy continuum operating on reproductive and ontogenetic rate shifts in evolution of selected character complexes.

Our biogeographical analysis of the ddRAD phylogeny explains the evolutionary history of Middle American heroine cichlids and demonstrates that biogeography is a much better indicator of evolutionary relationships in this fish group than are most morphological characters due to their ecological correlation. The two species-richest areas of Middle America (the Usumacinta and the San Juan river basins) are reconstructed as the two ancestral areas of the whole diversification of cichlids in Middle America. The majority of cichlid diversification in Middle America thus appears to be a striking case of localized *in situ* evolution. The two cichlid evolutionary centers in Middle America are very dissimilar in virtually all aspects of their cichlid evolution. These aspects include 1) the degree to which they have acted as faunal refugia, 2) their role in the colonization of the rest of Middle America, 3) their rate of faunal evolution, and 4) the degree of sympatric speciation and type of morphoecological divergence in their diversification. An interesting biogeographical discovery of the ddRAD phylogeny is the existence of an Isthmian clade grouping all amphiphine cichlids found in eastern Panama. The group is morphologically quite heterogeneous and dissimilar and a close relationship of these species was never before hypothesized.

In the Discussion of the study we summarize arguments demonstrating that the Middle American heroine cichlids are perhaps the most morphologically and ecologically diverse clade of Neotropical cichlids in that they show greater diversification of functionally significant morphological traits than comparable fluvial assemblages of South American or African cichlids. We further summarize the three main reasons for the past struggle in classifying the Middle American cichlids which are repeated evolution of ecomorphologies, reticulate evolution and (among one clade, the amphiphines, finally resolved in this study by the NGS phylogeny) additionally fast adaptive radiation.

Repeated evolution of ecomorphologies and adaptive radiations seem to be the main reasons for the unprecedented diversity of the Middle American heroine cichlid fishes but the whole diversity is clearly not the result of a single radiation because the Middle American heroines are not monophyletic, but are the result of two independent colonizations and further diversification within three clades. The three separate diversifications took place in only two limited areas and possibly in only two river basins of Middle America (in the Usumacinta and the San Juan ichthyological provinces) while the rest of Middle America is characterized by classic allopatric evolution (except for eastern Panama and the Panuco basin of Mexico which constitute likely two other smaller local diversifications). The herichthyine and astatheroïne diversifications are not true fast adaptive radiations because we see a slow gradual gain of diversity while fast adaptive radiation is only evident in the amphiphines. All three

diversifications however took place within the two limited geographical areas and thus likely included diversification in at least partial sympatry fulfilling the criterion of adaptive radiation (SCHLUTER, 2000; SCHLUTER & NAGEL, 1995). The three adaptive radiations in Middle American cichlids are ancient and now completed but on a smaller temporal and spatial scale we still have the chance to study active ongoing adaptive radiations in the small crater lakes of Nicaragua (BARLUENGA *et al.*, 2006; BARLUENGA & MEYER, 2004; GEIGER *et al.*, 2010, 2013; SCHLIEWEN *et al.*, 2006) and in the genus *Herichthys* with its polymorphic species and sympatric species-pairs (discovered in this study).

The only other example so far known among the Neotropical cichlids apart from the amphiphines that also fulfils all criteria of cichlid adaptive radiation are the two *Crenicichla* species flocks of the Uruguay and Iguazu/middle Parana river basins of SE South America (the latter discovered by our team; PIÁLEK *et al.*, 2012; BURRESS *et al.*, 2013). The diversification of the herichthyine and astatheroine cichlids is in their lack of rapid speciation more similar to the most widely publicized “adaptive radiation” in the Neotropical cichlids, that of the geophagine cichlids (LÓPEZ-FERNÁNDEZ *et al.*, 2010, 2013). Several crucial differences however still distinguish these diversifications and show that the herichthyine and astatheroines cichlids are much closer to the definition of adaptive radiation than are the geophagine cichlids. The differences are 1) the degree of ancestral sympatry (absent in geophagines, nearly absolute in MA cichlids), 2) the degree and geographical extent of sympatry of sister species (virtually absent in geophagines, very common in MA cichlids), and 3) the ecomorphological homogeneity of species within genera (virtually absolute in geophagines, not showing repeated evolution of ecomorphologies with most ecomorphologies are restricted to single genera, while ecomorphologies have evolved repeatedly among the MA cichlids). The diversification of the geophagines has been proposed as an important case of adaptive radiation in the Neotropics even though they do not fulfill its important criteria, i.e. rapid speciation (LÓPEZ-FERNÁNDEZ *et al.*, 2013 *contra* LÓPEZ-FERNÁNDEZ *et al.*, 2010) and sympatric or at least spatially limited evolution. The geophagine cichlids also do not qualify as a species flock (or multiple species flocks) which is the typical form of cichlid adaptive radiations. Our comparisons show that there is nothing special about the diversification of the geophagine cichlids which represent an ordinary evolutionary radiation and that Middle American cichlids include much better examples of adaptive radiations with a much higher degree of in situ (or even sympatric) evolution and much faster radiations (the amphiphines).

The Middle American cichlids are very different from the geophagines and from all other Neotropical cichlid clades. They are the only Neotropical cichlids that show so many times repeated evolution of ecomorphologies (classified here as 31 separate genera) and they are the only Neotropical cichlids with such a striking diversity pattern in terms of species/area relationships. The morphospace occupied by the Middle American heroine cichlids is comparable to that of the geophagines despite only ca 15% area available for the diversification of heroines in Middle America compared to the geophagines in tropical South America. When we leave aside the largest and most modified and diverse Neotropical cichlid genus (*Crenicichla*) which occupies about a half of the total morphospace of the geophagines (LÓPEZ-FERNÁNDEZ *et al.*, 2013) the Middle American heroine cichlids actually have the largest diversity of morphologies of all Neotropical cichlid clades (LÓPEZ-FERNÁNDEZ *et al.*, 2013). Nowhere else in the Neotropics can so many closely related yet ecomorphologically so different species be found within single small areas of endemism (river basins) in sympatry as in Middle America. There are two such diversity peak-areas in Middle America (in the Rio Usumacinta with ca 66.000 km² and in the Rio San Juan basin with ca 40.000 km²). Our biogeographical analyses additionally show that these two river basins are the evolutionary centers of the Middle American cichlid diversification (not just depositories or refugia of

diversity) and that the Middle American cichlid diversity is primarily derived from evolution in just two river basins.

Finally, we compare the Middle American cichlid diversification to the famous lacustrine radiations of cichlids in the Great Lakes of Africa with which the diversity of cichlid fishes in Middle America appears to have more in common than with most riverine cichlid assemblages. Among the Great African Lakes Lake Tanganyika (reviewed, e.g., in KOBLMÜLLER *et al.*, 2008) appears to be the most similar to the Middle American cichlid diversification based on three accounts.

Firstly, the Middle American cichlid diversification with ~ 120 valid species actually approaches the species diversity in Lake Tanganyika (the third most diverse lake after Malawi and Victoria with ~ 200 valid and ~ 250 estimated species) and surpasses radiations in all other lakes in Africa (lakes Kyoga and Edward/George have ~100 and ~ 60 species, respectively; TURNER *et al.*, 2001). Unlike the Lake Tanganyika cichlid fauna the cichlid fauna of Middle America is however not the result of continental-wide cichlid diversity but is the result of (two coincident) colonizations by just one cichlid clade, similarly to Lakes Malawi and Victoria in Africa.

Secondly, the large species diversity in both ecosystems (Middle America and Great Lakes of Africa, especially Lake Tanganyika) appears to be due to diversification by natural selection of feeding ecomorphologies. The tribes of Lake Tanganyika cichlids and the genera of Middle American cichlids are limited to particular ecological niches. The genera of Lake Tanganyika cichlids and of Middle American cichlids are thus primarily ecomorphologically delineated. The repeatedly evolved ecomorphologies (between but also within the three main clades) in Middle American cichlids which we classify as genera are thus to a large extent equivalent and comparable to repeatedly evolved ecomorphologies (also classified as genera) between (and also within) the tribes of the Tanganyikan cichlids. The spectrum of ecomorphologies in Middle America is of course much lower than in Lake Tanganyika (lacking e.g. the very specialized scale-eaters or mouthbrooders) but it is surprisingly high for riverine cichlids. The Middle American cichlid diversification lacks all the pelagic ecomorphologies (e.g. predators-piscivores as in Hemibatini and Bathybatini, pelagic plankton-feeders as in Trematocarini, Benthochromini, or Cyprichromini) for obvious limitations of the riverine habitats but the shore-dwelling Lake Tanganyika cichlid diversity is rather comparable to the Middle American riverine cichlid diversity which WINEMILLER *et al.* (1995) found as the most diverse riverine cichlid faunas both in Africa and in the Neotropics. The shore-dwelling Lake Tanganyika cichlids include several scraping groups associated with rocky shores (Eretmodini, Tropheini, Ectodini), sandy/muddy bottom detritivores/sifters (e.g. Limnochromini) and also predators/piscivores (e.g. Boulengerochromini, Cyphotilapiini, Lamprologini) and all these main ecomorphs are also very common and have repeatedly evolved in the Middle American cichlids.

Thirdly, while none of the two diversifications (Lake Tanganyika and Middle America) is monophyletic they are both composed of several independent invasions that were followed by diversification in a circumscribed biogeographical setting, the Lake Tanganyika cichlids in a lacustrine environment and the Middle American cichlids in only two drainage basins. We argue that the three Middle American parallel diversifications are primarily riverine diversifications in two drainage basins, but a lacustrine diversification in the geological past cannot be at present excluded based on scant geological knowledge.

Several authors suggested (reviewed, e.g., in SEEHAUSEN, 2006) that cichlid radiations are usually associated with truly lacustrine conditions. Several indications from the Neotropics (especially the *Crenicichla* species flocks of the Iguazu and Uruguay rivers) including the presented results for the Middle American cichlids and from Africa however suggest that only the most spectacular and species-richest cichlid radiations are truly lacustrine in origin.

Smaller radiations are now known from both smaller lakes and from riverine habitats. The diversified riverine haplochromine (serranochromine) fauna in southern Africa has been suggested to have possibly originated in the paleo-Lake Makgadikgadi (JOYCE *et al.*, 2005), but this argument was heavily based on this supposition that cichlid radiations are usually associated with lacustrine conditions. Our results weaken this association between cichlid radiations and lacustrine conditions.

Part IV. Future directions

I have recently started a much larger number of cooperative projects (see CV) than was the case in my previous research. The need for this came mostly from my ever decreasing amount of time and also the ever widening scope of my projects.

The most promising widely important specific topic for future studies are our speciation projects as outlined above. These projects will also see most of methodological improvements and innovations in our lab. This is one of the few fields of our research that will also have an experimental part in aquarium conditions (in our recently rebuild aquarium facility) in which we will be testing phenotype stability in species flocks under standard conditions, phenotype-environment correlation and trait utility, and also divergent selection in experiments with pure species and with artificially bred F1 hybrids. Our speciation projects have gained momentum due to the passion for genomics and a postdoc grant to Lubomír Piálek who will lead these efforts in our lab with my guidance providing ichthyological and contextual analyses. These speciation studies have recently been shifted to an internationally cooperative effort with a US based research group working on the same topic in the same taxa.

On larger scales and scopes we will be aiming at completing the large-scale phylogeny and large-scale evolution of the vast majority of Neotropical cichlid species in the Neotropics. A large portion of this topic to which I am now shifting most of my attention (while my postdoc and PhD students pursue mainly the speciation studies or other projects outlined here) is cichlid evolution in the western Amazon in the setting of where the Andes meet the Amazon focusing on the large-scale biogeographical remodeling of the drainage patterns and ecological mosaics of the western Amazon caused by the elevation and west-east propagation of the Andes.

On a more general level we will be pursuing studies on comparative biogeography of cichlid and non-cichlid freshwater fish groups (currently financed by a postdoc grant) and other animal and plant groups in the Neotropics with considerations of ecological, paleo-climatological and geomorphological aspects.

Our studies will remain diversified thematically as outlined in the presented thesis and will see a continuation of strong emphasis on primary and descriptive studies of biodiversity including further discoveries and descriptions of new taxa and new biogeographical records.

As a morphologist my holy grail would be to investigate the evolution of morphological characters with the inclusion of their ontogenetic development and transformation. This is a nearly utopic dream because it is (1) extremely difficult to get the necessary material (the ontogenetic series would have to be for all species collected in the wild, unlike the ontogenetic data for the much more environmentally conservative coloration pattern characters which I did obtain from aquarium-bred specimens) and (2) a project of this scope and with this theme is outside the possibilities of obtaining any financial funding. Despite this I am convinced that it would greatly enhance our understanding of morphological evolution and the origin of especially adaptive morphological characters, especially in combination with the massive revolution from genomics. Something of this scope and importance has never

been done for any freshwater group of fishes as far as I know. There is however a chance for this research theme to become realized in our speciation studies on selected smaller groups, e.g. the *Crenicichla* species flocks or the major lineages of Middle American cichlids by combining the cases where one could collect ontogenetic series in the wild with aquarium bred ontogenies.

I personally will also continue with the exploration and search for those most ultimate explanations of the Neotropical biodiversity on the cichlid and freshwater fish model and in comparative studies with other elements of the Neotropical biodiversity. While many of my papers feature a set of both proximate and ultimate explanations all of these are still to a greater or smaller extent rather descriptive and/or narrowly focused on just the studied fish group(s). The most ultimate explanations in evolution of biodiversity are in general planetary scale forces caused (1) to a large extent by extraterrestrial forces governing the movements of planets inside the Solar system on periodical and non-periodical pathways and (2) due to the inner geological dynamics of the planet Earth. These together shape the shape and positions of the continents determine their overall climate and distribution of major biomes and life zones and thus ultimately shape the course of evolution and shape biodiversity patterns across the biotas. While the most ultimate explanations as drivers of evolution are those hardest to study and hardest to prove (following the maxim that extraordinary claims require extraordinary evidence) they are also by far the most interesting and fundamentally important with wide scale generality.

In the example of Middle American cichlid diversification treated in the above study their evolution in just two main river basins seems to have been forced by external geological forces because large-scale volcanism seems to have caused their almost complete extinction in the intervening area between the two centers of diversification (this hypothesis is based on the extremely good temporal and spatial agreement between the volcanism and the reconstructed cichlid extinctions in their genomic phylogeny; Řičan et al., in prep.). The impulse for their localized diversification thus could have been this large scale extinction event. At the same time this extinction event was caused by the same geological force that connected the island archipelago of developing Middle America into a single land bridge. Hence colonization of lower Central America from the north was enabled by the same geological forces as have triggered both their partial extinction and their explosive localized radiations.

The unparalleled diversification of the Middle American cichlids per-se that is so different from the majority of South American Amazonian cichlids (as again first demonstrated in the central study of this thesis outlined above) is based on indirect evidence, deductions and correlations (Řičan et al., in prep.) also the result of geological forces and areal configurations. Middle America has been for most of its history a semi-isolated island archipelago with limited dispersal capabilities for most strictly freshwater fish groups. Despite its 3-10 Ma long recent connection with both North and South America the freshwater fish fauna of Middle America still has signatures of island biodiversity patterns. These are characterized by impoverished diversity of main faunal clades otherwise found on the surrounding continents, but compensated by unprecedented diversification of some of its faunal elements (in Middle America including the cichlids). Madagascar and the Great Antilles are the prime examples of this general rule, and Middle America as well. Since most strictly-freshwater fishes could not reach (and did not reach) developing Middle America for long tens of millions of years the faunas that managed the colonization (including the cichlids due to their marginal degree of marine water tolerance) have evolved and diversified in a biologically less competitive setting than was present in the surrounding continents and thus have realized niches otherwise occupied by other earlier or more successful groups (such as the characoid and siluroid fishes in South America which did reach Middle America only after cichlid diversification was

already completed). What we are thus seeing in Middle American cichlids is a realized potential that in South American cichlids due to competition with other fish groups remained unrealized. The ultimate biological explanations in this case are thus different colonization capabilities (paper 17) and group or faunal selection in South America which was almost completely lacking in Middle America due to its prolonged semi-isolation.

Variations of this theme of lack of group selection caused by physical barriers can also be found in the opposite end of the Neotropics in southern tropical South America where we have for example found again unprecedented diversification of cichlids (this time in the genus *Crenicichla*) again in settings with reduced competition from other fish groups, this time due to restricted dispersal and semi-isolation caused by rivers full of waterfalls and rapids, which are in turn a direct result of the underlying geology, which in turn results and dates back to large-scale volcanism responsible for the continental drift between South America and Africa in the Mesozoic (long before most modern fish groups have actually started to diversify).

My ultimate goal in the cichlid projects is thus to gain a multidimensional understanding of their evolution which would constitute a series of large monograph papers and one day a book that would interconnect all the facets of the whole of Neotropical cichlid diversification and evolution in a continental, ecological and biodiversity context in one cohesive natural history compendium.

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Part V.

Complete list of publications

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Part VI.

Monograph paper:

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