Phylogenetic, morphological and systematic studies of three genera of Cichlidae (Teleosts fish, Perciformes) endemic of Madagascar

Abstract
The aim of this research is to know the degree of reliability of the classification method based on molecular studies versus those based on the morphological characters. The hypothesis is as follows: the results from these two methods would be different. Eleven species belonging to the three genera of endemic Cichlids emerge from the genetic study using a mitochondrial DNA fragment COI, and four of which are new species for Science: Paratilapia sp. Nosy Be, Paratilapia sp. smallspots Est, sp. Nosy beet Paretroplus sp. Lokoho. Morphological studies of 45 characters from 42 specimens did not correctly classify the three genera. Molecular systematics is thus a more reliable and informative method for studying the evolution of a given taxon and the link between its members.

Keywords: IOC DNA, Cichlidae, Madagascar, morphometry, phylogeny, systematic

Introduction
Malagasy continental waters are recognized for their richness in biodiversity.¹ Up to 41% of Madagascar’s freshwater fish species (71 out of 172) are endemic, with two endemic families.² Despite the fact that Madagascar is one of the conservation priority countries,³ its freshwater fish are still subject to several pressures, such as the introduction of carnivorous fish species, the destruction of forests, overfishing, habitat transformation for other human uses, and lack of knowledge about fish.⁴ Available information on the island’s native freshwater fish is very low, although it is extremely threatened.⁵ Most research on these Malagasy freshwater fish is now outdated.⁶,⁷ Malagasy fish have been poorly studied because of the difficulty of conducting sampling.⁸ In short, minimal information and current knowledge on the systematics and evolution of freshwater fish from Madagascar exists, including some genera of Cichlids. The family Cichlids is one of the richest among all vertebrates.⁹ This family is mainly found in Central Africa, Latin America, Madagascar and some native species in India and the Middle East.¹⁰ The Cichlidae family forms a monophyletic group and is subdivided into four subfamilies: Etroplinae (Indian and Madagascar), Ptychochromine (Madagascar), Cichlinae (Neotropical Region) and Pseudocrenilabrinae (African), according to the classification derived from molecular and morphological data.¹¹

However, the pressures on these genres continue to grow. Knowledge of diversity, in particular species richness, is a basic element to better understand biodiversity and its conservation. The present work, which focuses on morphologic and genetic studies of some Cichlid species endemic to Madagascar, intends to enrich the knowledge of Malagasy ichthyological fauna.

The hypothesis of this research is as follows: “The classification resulting from the genetic study would be different from that resulting from the morphological study”. Many authors such as Dayrat¹² have proposed an integrative approach to taxonomic study. It is generally used to distinguish between cryptic species or to interpret an infraspecific variation. The main objective is to know the degree of reliability of the method of classification of the species operating the external characters (morphological and phenotypic) compared to the method of genetic classification; or (1) to know the phylogenetic relationships of the species belonging to the three endemic genera of Cichlidae of Madagascar: Paratilapia, Ptychochromis and Paretroplus, (2) to draw up the classification based on the external characters, (3) to identify the similarity and/or the difference results from the two classification processes; and (4) determine the main morphological characters that distinguish the groups studied.

Materials and methods

Construction of the genetic tree
A small portion (corresponding to 1g) of the pectoral fin of each individual of the studied species each kept in a tube containing 90% alcohol serves as a genetic sample for the molecular study.

Total genomic DNA was extracted from tissue samples using proteinase K digestion at a concentration of 10mg/ml, followed by a standard salt extraction protocol according to Bruford et al.¹² A mitochondrial DNA fragment of approximately 700 base pairs of Cytochrome Oxidase I (COI) was amplified using the primers developed by Hebert et al.,¹³ Ward et al.,¹⁴ and Ivanova et al.,¹⁵,¹⁶ PCR (Polymerase Chain Reaction) products were separated on an automated ABI 3130XL DNA sequencer at the Technical University of Braunschweig in 2012 and ABI 3730 at the Ludwig-Maximilians University of Munich in 2013.

The CLC Main Workbench 6.8.2 software was used for quality control of the DNA sequences and the sequences were aligned using the MEGA or Molecular Evolutionary Genetics Analysis software version 6.0.¹⁷ A search for the identity of all sequences studied and aligned was carried out in “GenBank”. An NCBI nucleotide blast online search (http://www.ncbi.nlm.nih.gov/) Followed by an insertion of the sequence to be analyzed was then performed. The result of this search gives the species closest to that analyzed with a precision of the similarity rate between them; the sequence of this one was used for the construction of the phylogenetic tree. A total of 9 GenBank sequences were added to the 42 identified DNA sequences. The fasta
file of the result of the MEGA alignment is opened with the software j-Model test 2.1.7. Its principle is to have a AICc equal to 0.18. The Mr Bayes 3.2 software is then used to obtain a consensus tree, with a parameterization of 2 million generations (bootstrap method), a sampling frequency equal to 100 and an ignorance rate of 0, 25. The final result obtained from Mr Bayes, in the form of a nexus file, was opened with the Figtree software version 1.4.2., In order to know the degree of link existing between the species studied is known.

**Classification based on external characters**

The Dyer & Chernoff method was applied to the entire body of captured fish specimens. The choice of characters to be observed was based on the characteristics of the family of Cichlids proposed by Paugy et al. In total, 45 characters were studied. An encoding of each character state has been done, taking into account the character state that is primitive. The latter has been coded with 0, and the coding evolves according to the derivation of the state of the characters. The matrix thus obtained has been processed in a specific online software (http://www.genomes.urv.cat/UPGMA/index.php.). The DendroUPGMA (Vallve et al., 1999). For this study, the coefficient used is the RMSD or Root-Mean-Square-Distance to evaluate the statistical significance of the difference between two values. The resulting bit was inserted later in FigTree version 1.4.2.

**Identification of morphometric and meristic differences**

**Measurement and counting:** the 26 parameters to be measured refer to those proposed by Paugy et al. for studies of Cichlids, then adapted according to the needs of the author: 1-diameter of the eye (dO), 2-height of the anal fin (HA), 3-height of the body (HC), 4-dorsal fin height (HD), 5-pectoral fin height (HPC), 6-height caudal peduncle (HPC), 7-pelvic fin height (HPi), 8-head height (Ht), 9-length of the anal fin base (LA), 10-mouth length (LB), 11-length of the dorsal fin base (LD), 12-length of the fin base pectoral (LPC), 13-length caudal peduncle (LPC), 14-pelvic fin base (LPi) length, 15-postorbital length (LpostO), 16-length preanal (LPreA), 17-length predorsal (LPreD), prepelvic 18-length (LPreO), prepectoral 19-length (LPrePc), prepelvic 20-length (LPi), standard 21-length (LS), total 22-length (LT), length of the head (LH), 23-mouth width (IB), 24-body width (IC), 25-lar interorbital gland (IO), 26-width of the head (lt).

Measurements on the lateral face and in front view were made using a calipers D20TN Mitutoyo 200mm in length and the accuracy is 0.02mm.

Four distances between the insertions of the different fins were calculated after obtaining the values during the measurements: (a) the distance between the pelvic fin and the anal fin (dPIA); (b) the distance between the pectoral fin and the anal fin (dPcA); (c) the distance between the pectoral fin and the pelvic fin (dPePi) and (d) the distance between the dorsal fin and the anal fin (dDA).

For this study, counting with the naked eye or a binocular loupe, fin (dorsal and anal) and scales along the longitudinal, lateral and operculum lines was performed.

**Statistical analysis:** statistical analyses were performed on SPSS or Statistical Package for the Social Sciences version 17.00 to analyze the data from the study of morphometric and meristic parameters.

For this study the Mann-Whitney test was performed using relative values for morphometric parameters (variable parameters as a function of age) and exact values for meristic characters. PCA was used to describe the variables characterizing each individual. It makes it possible to combine the variables studied in the form of factors (reduction in the number of variables) and to extract all the factors by keeping only those (containing the principal components) which are the most influential. Twenty localities have been collecting endemic Cichlid specimens from 2011 to 2014.

**Results**

**Molecular systematics**

The result of the genetic analysis of the 51 COI mitochondrial DNA sequences of which 42 obtained in this study and nine, downloaded from GenBank, each containing 572 bases (sites), can be summarized by the phylogenetic tree with a standard average value of the frequency equal to 0.004496 (Figure 1). *Pachypanchax omnolotus* (*Apoplocheilidae*) collected in Lake Amparibihie served as an external species for the study.

---

**Figure 1** Phylogenetic tree of 11 endemic species of Malagasy Cichlids. RDM Sequences of Dr. Roger Daniel Randrianaiana; sequences A01, A02, A09, B7 and ZCMV of Prof Dr. Miguel Vences; AF and HQ sequences from GenBank.

**Paratilapia**: a monophyletic genus, is thus a sister genus of *Psychochromis*. *Paratilapia* sp. smallspots paired with *Paratilapia* sp. East and *Paratilapia* sp. Manombo is a sister species to the other two species of *Paratilapia*. Thus the smallspots species of the East is different from the largespots species of the North. For northern species, individuals *Paratilapia* sp. Nosy Beet those of *Paratilapia pollenis* form two populations of different species, a difference maintained at 86%.

**Psychochromis**: is a monophyletic genus. *Psychochromis oligacanthus* is a sister species of the other two *Psychochromis loisellei* and *Psychochromis grandidiieri* combined. *Psychochromis oligacanthus* is composed of populations of two different species: *Psychochromis oligacanthus* ss and *Psychochromis* sp. Nosy Be with a sustained difference of 89% in P.

**Paretroplus**: is also a monophyletic genus. *Paretroplus loisellei* is
a sister species of the other three *Paretroplus* species (*Paretroplus polyactis*, *Paretroplus* sp, Lokoho and *Paretroplus maculatus*). Two species of *Paretroplus polyactis* sl, different to 100% (in F), result from this study: *Paretroplus* sp. Lokoho and *Paretroplus polyactis* Maroantsetra. The latter is paired with *Paretroplus polyactis* North of the “GenBank”.

**Morphological systematics**

A total of 45 external characters were studied on each of the 42 fish specimens and obtained the dendrogram of Figure 2. The genera *Ptychochromis* and *Paretroplus* are sister groups and these two groups combined form a sister group of the genus *Paratilapia*.

**Paratilapia**: is a monophyletic group and *Paratilapia pollenii* sl is a cryptic species. Antsahamelyoka harbor two types of populations of *Paratilapia polleniss* with different characters (polymorphic); and the population of *Paratilapia* sp. Nosy Be (Amparibibe), in spite of their genetic difference with that of *Paratilapia polleniss* Amparibibe, presents a great similarity of the characters studied.

**Ptychochromis**: belongs to a monophyletic group and is a cryptic genus. The four species are not monophyletic groups. Individuals of *Ptychochromis grandidieri* Amboditandroho form a sister group of all other individuals of the genus *Ptychochromis* (in E). Thus, the individuals of *Ptychochromis grandidieri* differ in their characters. Also, referring to node N, *Ptychochromis* sp. Nosy Be and *Ptychochromis oligacanthus* ss Amparibibe (Nosy Be) show morphological differences. The individual of Pt. *Loisellei* RDR 1213 has characteristics that differentiate him from all other individuals belonging to this species.

**Paretroplus**: is a monophyletic group. It is the same for the species *Paretroplus loisiellii* which is a sister species of the three others combined. Individuals of *Paretroplus polyactis* Pangalanene can be divided into several batches of individuals with different characters; it is the same for *Paretroplus maculatus* Antsif. Thus, *Paretroplus polyactis* and *Paretroplus maculatus* are both paraphyletic groups (in J).

**Inter and intra group differences**

According to the results of the U test carried out for the genera *Ptychochromis* and *Paretroplus*, the number of individuals is respectively *n* =15 and *n* =16 and for *α*=0.05, the value of *U* =44. In total, 23 parameters out of the 37 studied make it possible to distinguish the two kinds, a 65% difference. Thus, *Paratilapia* is distinguished from *Ptychochromis* by these main characters: a larger body, a larger head, a longer and wider anal fin, a longer caudal peduncle and fewer rays of the dorsal fin.

For the second group compared, the genera *Ptychochromis* and *Paretroplus*, the numbers of the individuals are respectively *n* =15 and *n* =16 and for *α*=0.05, *U* =70. According to the statistical results, 15 characters out of the 37 studied are different between them. The body, the fins and the meristic characters are the main differentiations of these genera.

The result from the U test shows that for *Paratilapia* (at 11 individuals) and *Paretroplus* (at 16 individuals) and for *α*=0.05, *U* =47. *Paratilapia* differ of *Paretroplus* of 14 morphological characters out of the 37 studied. As for *Paretroplus*, it is distinguished from *Paratilapia* partly: by a higher body height, the rays of the dorsal fin and the anal fin more numerous and more scales on the lid and along the longitudinal and lateral lines higher.

The main morphometric and meristic differences of *Paratilapia pollenii* ss and *Paratilapia* sp. Nosy Be remains especially in the insertion of the pelvic fin and the height of the dorsal dorsal.

Species of the genus *Ptychochromis* differ mainly on the characters of the head. *Ptychochromis loisiellii* and *Ptychochromis grandidieri* show no significant difference.

For the genus *Paretroplus*, the difference varies according to the species compared: *Paretroplus loisiellii* and *Paretroplus polyactis* are 84% different for the 37 parameters, whereas *Paretroplus loisiellii* and *Paretroplus maculatus* show no significant difference (Figure 3).

---

**Figure 2** Phylogram from the 45-character study of 42 individuals.

**Figure 3** Bivariate Scatterplot of specimens of 10 species according to the two regression factors.

---

**Citation**: Ranalison O, Mampianina RL. Phylogenetic, morphological and systematic studies of three genera of Cichlidae (Teleosts fish, Perciformes) endemic of Madagascar. Biodiversity Int J. 2018;2(6):538–543. DOI: 10.15406/bij.2018.02.00110
Phylogenetic, morphological and systematic studies of three genera of Cichlidae (Teleost fish, Perciformes) endemic of Madagascar

Out of twenty localities collecting endemic Cichlid specimens for three years from 2011 to 2014. The following points are worthy of mention.

Identities of the studied species

Several studies have shown that the subfamily Eropilinae (including the genus Paretroplus) is sister to Psychrochromini (composed in part by Paratilapia and Ptychochromis) plus African Cichlids and Neotropical.10,23–25

Concerning the genus Paratilapia, this study made it possible to distinguish three species: P. poleni ss the typical form and two other new species: P. sp. Nosy Be (Amparihibe) and P. sp. smallspots Est. The first species, P. poleni ss or P. poleni “largepots” corresponds to that described by de Rham & Nourissat26 to name individuals with large points and having a distribution in the northern part of Madagascar. The characterization of this species as “sensus stricto” or typical is first justified by the Andapa specimens with large dots and which are of the same type as those of Sambirano;26 a locality where the holotype described by Bleeker in 1868 was captured. The second species, P. sp. Nosy Be having big points is according to the bibliography and the results of “GenBank”, a species of P. poleni “largepots”. However, the genetic tree from this study to see the links between species shows that this species differs from P. poleni ss to 86%. It should be noted that all these individuals were collected in Lake Amparihibe. In 1882, Sauvage27 described the species P. bleeeri, a species also having large dots but collected in Antananarivo. This description is described as “hasty” by many authors26,28 and is considered invalid even though Loiselle & Stiasnys29 adopted this nomenclature. As a result, no other description after that of Bleeker has been made, thus the second species resulting from this study P. sp. Nosy Be is a still undescribed species and is a “new species”. Lake Amparihibe is home to two different populations of Paratilapia “largepots” including the species P. sp. largepots Nosy Be who is absent in Andapa. The third species, P. sp.smallspots Est is considered another species after Rham & Nourissat,26 but as P. poleni East by “GenBank”. The accuracy of the origin of this species is accurate considering that the places of collection of our specimens were the Eastern part of the Island (case of Nosy Be) and the South-East part (case of Manombo). Other authors26 confirm that it really occupies these localities. In 1878, Bleeker described the species Paracara typus Est, which was later modified into Paratilapia typus11 and has a strong resemblance to P. sp. smallspots Is but whose origin of the only exemplary type is unknown. Indeed, P. sp. smallspots Est could be synonymous with that described by Bleeker or a new species that is not yet described.

For Ptychochromis, it is composed of four species: Pt. Loisellei, Pt. Grandidiieri, Pt. Oligacanthus and Pt. Sp. Nosy Be. Stiasnys & Sparks25 described the species Pt. Loisellei, formerly named Pt. Sp. Northeast Garaka. Pt. Loisellei being genetically closer to Pt. Grandidiieri spreading also to the East, and further north. Pt. Oligacanthus is so named because: the holotype that Bleeker described in 1868 came from Sambirano and even though our specimen was collected in Nosy Be, it is 100% identical to that of GenBank from Mananjeba, a very locality close to the typical locality, and whose two basins touch each other; furthermore, Sparks25 states that populations in the north possess the characters described by Bleeker.31 Apart from this first collection of the holotype, another from Ampobihava Lake (Nosy Be) was, at the same time, described by Bleeker.31 Sparks32 assumes that Pt. Sp. Mananjeba and Pt. Sp. Nosy Be are of the same species but Loiselle34 finds a difference between the population of Nosy Be and that found on Grande Terre. He also adds that each lake in Nosy Be is populated by populations of different species that have diverged from a common species. As the molecular analysis shows a 100% difference between the Nosy Be and Mananjeba species and a 100% resemblance of the species Pt. Sp. Nosy Be with that of Nosy Be. In addition, Loiselle34 discovered formally the non-validity of the specific name Pt. nossibeensis proposed by Guinane,33 this species Pt. sp. Nosy Be is then undescribed and is a “new species”. Thus, this study made it possible to elucidate the doubts about the belonging of Pt. De Mananjeba and that of Nosy Be to the same species.26

Martinez & Sparks29 confirms the results on the links between species of the genus Paretroplus. Paretroplus polyactis sl and Paretroplus loisellei are the two species belonging to this genus, found in the eastern part of Madagascar. However, according to the phylogenetic tree obtained, Paretroplus maculatus is closer to Pe. polyactis. Thus, the statement that Pe. polyactis is the only genus occupying exclusively the eastern part and is in a clad apart from all other Paretroplus,7 has not been confirmed. P. polyactis Maroantsetrae and Pe. sp. Lokoho are, according to genetic studies, 100% different species despite their same distribution in the eastern part of Madagascar. According to the result of GenBank, Pe. Maroantsetra polyactis is 100% identical to Pe. polyactis in the North. The explanation of the classification choice is that the holotype of Pe. polyactis described by Bleeker in 1879 comes from the Canal des Pangalanes. Loiselle,37 due to the difference in color, suspects the presence of 2 or more Pe species. polyactis. Thus, a probable speciation within this species would have occurred in this northeastern part of the island. As a result, the species Pe. sp. Lokoho is also a “new species” (Figure 4).

Figure 4. Species studied (Photos Randrianiaina, 2013 and Nourissat, 2006) (a) Paratilapia polleni (b) Paratilapia sp. smallspots Est. (c) Ptychochromis grandieri. (d) Ptychochromis loisellei. (e) Ptychochromis oligacanthus ss -E: Scale. (f) Ptychochromis sp. Nosy Be. (g) Paretroplus sp. Lokoho. (h) Paretroplus maculatus. (i) Paretroplus loisellei. (j) Pachypanchax omanotilus.

Citation: Ranalison O, Manpiana RLA. Phylogenetic, morphological and systematic studies of three genera of Cichlidae (Teleost fish, Perciformes) endemic of Madagascar. Biodiversity Int J. 2018;2(6):538–543. DOI: 10.15406/bij.2018.02.00110
Phylogenetic, morphological and systematic studies of three genera of Cichlidae (Teleosts fish, Perciformes) endemic of Madagascar.

Similarities and differences between molecular systematics and morphological classification

The three genera have been well separated on the dendrogram of characters and each possess their respective clade. Although the three genera are monophyletic groups, on the phylogram and statistically, the genera Ptychochromis and Paratilapia are more distant and have the most difference. This is opposed to molecular results. With this difference of results, the following hypothesis could be considered “the phenotypically similar characters can be genetically different” knowing that different codons can code for the same protein and all of these proteins are the phenotypic image reflecting the genotype of an individual. The fact that Ptychochromis and Paretoplus, like Paratilapia and Paretoplus, show a difference over almost all the meristic characters, would be a justification for the belonging of the two genera (Ptychochromis and Paratilapia) to the subfamily Ptychochrominés. In addition, Paretoplus found in the subfamily Eritropilae is distinguished from the other two genera by the height of its body and belongs to the clade of high bodies. 

With regard to the genus Ptychochromis, Pt. lawellei and Pt. grandidieri being more genetically similar and morphologically identical (no significant difference in traits between these two species was observed in the Mann-Whitney test result). Stiassny & Sparks confirm that the similarity of these two species is very high. Finally, the two species Paretoplus maculatus and Pe. polyactis are more closely related genetically and on the phylogram. According to the ACP, the individuals of Pe. sp. Lokoho are isolated from those of Pe. polyactis, which confirms the result of the molecular study showing the belonging of these individuals to 2 different species.

Thus, if it is necessary to have an idea about the distributions at the genus level, the morphological classification is still instructive. But in the case where the details of the systematics, ie the degree of relations between taxa (at the genus and especially inter and intraspecific level) and the direction of evolution, the genetic method is reliable and more informative. Ambiguities or differences in morphological classification, especially for cryptic species, are solved by the genetic method with a precision of the relationship. This finding has also been observed for other classes of vertebrates.

Also, the morphological differentiations do not necessarily translate into molecular speciations. In addition, although many informative biogeographic studies have been conducted many times, ambiguities about classification are not always resolved. Like the case of Paratilapia polleni, several more names are attributed to it and it is a complex species that needs a systematic revision. However, during this study, molecular genetics allowed to discover new species, a great asset that would not emerge from the morphological study. In sum, four (04) new species still undescribed spring from this phylogenetic study: Paratilapia sp. smallspots Is, Paratilapia sp. largespots Nosy Be, Ptychochromis sp. Nosy Be and Paretoplus sp. Lokoho.

In short, the hypothesis of this study is accepted; a significant difference exists between molecular systematics and morphological classification. This difference has appeared from the genus level and is more accentuated at the species level with a confirmed reliability of the genetic method. The result demonstrates that the combination of genetic studies and phenotypic diversity provides robust information on intraspecific distribution that will subsequently be a fundamental element for future conservation projects.

For the Nosy Be Lakes, researchers believe that the large satellite island of Nosy Be being virgin of non-native predators, would be one of the highest conservation priorities. Conservation actions include projects for the rational management of lake waters, especially for Lake Amparihibe, many conspecific species are observed in this study. After this study, the observation of several endemic and new species occupying these lakes and subsequent research that deserve to be carried out are additional arguments for conserving these lacustrine ecosystems of Nosy Be.

Thanks

We extend our gratitude to the Volkswagen Foundation for funding this study. We thank Professor Noromalala RAMINOSOA, Dr. Roger Daniel RANDRIANIANA and Dr. Fanomezana RATSOAVINA for their invaluable help. We also thank Professor Dr. Miguel VENCES for giving us the sequences of the fish he has collected to complete this study and Mr. Guy TAM HYOK for having agreed to collaborate during the collection of samples in the APPA center in Andapa, Madagascar.

Acknowledgements

None.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Eloaard JM, Gibon FM. Eco-hydrostéisme. In : Eloaard JM, Gibon FM, editors. Biodiversité et biologie des eaux continentales de Madagascar. IRD; 2001. p. 37–51.
2. Froese R, Pauly D. Fishbase. World Wide Web Electronic Publication; 2017.
3. Mittermeier RA, Myers N, Thomsen JB. Biodiversity hotspots and major tropical wilderness areas: Approaches to setting conservation priorities. Conservation Biology. 1998;12:516–520.
4. CAMP. Rapport version finale sur les poissons d’eau douce pour l’évaluation et plans de gestion pour la conservation de la faune de Madagascar. 2002; 448 p.
5. Sparks JS, Stiassny MLJ. Les poissons d’eau douce. In Betsch JM, editors. Perspectives naturelles et biodiversité de Madagascar. 2008; p. 283–310.
6. Stiassny MLJ, Raminosoa N. The fishes of island waters of Madagascar. Biological diversity in African fresh- and brackish water fishes. Geographical overviews, Symposium PARADI. Annales du Muséum royal de l’Afrique centrale, Sciences zoologiques. 1994;275:133–149.
7. Sparks JS. Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae). Molecular Phylogenetics and Evolution. 2004;30(3):599–614.
8. Nelson JS. Fishes of the world. 4th ed. Hoboken, New Jersey. 2006; 601 p.

Citation: Ranalison O, Mampianina RL. Phylogenetic, morphological and systematic studies of three genera of Cichlidae (Teleost fish, Perciformes) endemic of Madagascar. Biodiversity Int J. 2018;2(6):538-543. DOI: 10.15406/bij.2018.02.00110
Phylogenetic, morphological and systematic studies of three genera of Cichlidae (Teleosts fish, Perciformes) endemic of Madagascar

9. Genner MJ, Seehausen O, Lunt DH, et al. Age of cichlids: new dates for ancient lake fish radiations. Mol Biol Evol. 2007;24:1269–1282.

10. Sparks JS, Smith WL. Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). Cladistics. 2004;20(6):501–517.

11. Dayrat B. Towards integrative taxonomy. Biol J Linn Soc. 2005;85(3):407–415.

12. Bruford MW, Hanotte O, Brookfield JFY, et al. Single locus and multilocus DNA fingerprint. In: Hoelzel AR, editors. Molecular genetic analysis of populations: a practical approach. IRL Press, Oxford, United Kingdom: 1992; 225–270.

13. Hebert PDN, Penton EH, Burns JM, et al. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptesfulgerator. Proceedings of the National Academy of Sciences, USA. 2004;101(41):14812–14817.

14. Ward RD, Zemlak TS, Innes BH, et al. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London. Philos Trans B Soc Lond B Biol Sci. 2005;360(1462):1847–1857.

15. Ivanova NV, deWaard JR, Hebert PDN. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes. 2006;6(4):998–1002.

16. Ivanova NV, Zemlak TS, Hanner RH, et al. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes. 2007;7(4):544–548.

17. Tamura K, Stecher G, Peterson D, et al. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 2013;30(12):2725–2729.

18. Akaïe H. A new look at the statistical model identification. IEEE Transactions on Automatic Control. 1974;19(6):716–723.

19. Dyer BS, Chernoff B. Phylogenetic relationships among atheriniform fishes (Teleostei: Atherinomorpha). Zoological journal of the Linnean Society. 1995;117(1):1–69.

20. Paugy D, Lévêque C, Teugels GG. The fresh and brackish watershies of West Africa. Tome II. 2007. 527 p.

21. Scherrer B. 1984. Biostatistique. Gaëtan morin. 850 p.

22. Hair JF, Anderson RE, Tatham RL, et al. Multivariate data analysis. 5th edn. Prentice-Hall International, Upper Saddle River; 1998.

23. Stiassny MLJ, Chakrabarty P, Loiselle PV. Relationships of Madagascar cichlid genus Paretroplus, with description of a new species from the Betsiboka River drainage of northwestern Madagascar. Ichthyological Exploration of Freshwaters. 2001;12(1):29–40.

24. Friedman M, Keck BP, Dornburg A, et al. Molecular and fossil evidence place the origin of cichlid fishes long after Gondwana rifting. Proc R Soc B. 2013;280(1770):20131733.

25. Mc Mahan CD, Chakrabarty P, Sparks JS, et al. Temporal patterns of diversification across global cichlid biodiversity (Acanthomorpha: Cichlidae). PLoS One. 2013;8(8):e71162.

26. de Rham P, Nourissat JC. The endemic Cichlids of Madagascar. International Standard Bask Number, Spain, Barcelona. 2004;191 p.

27. Sauvage H. Histoire naturelle des Poissons. Collection Histoire, physique, naturelle, politique de Madagascar. Famille des Chromidés; 1891; p. 437–441.

28. Pellegrin J. Contribution à l’étude anatomique, biologiques et taxonomiques des poissons de la famille des Cichlids. Mémoires Société Zoologique. 1904;16:11–400.

29. Loiselle PV, Stiassny MLJ. How many Marakely? Cichlid Association 157: 2–8. Request For Comments. 1993;137:16–28.

30. Reinthal PN, Stiassny MLJ. The freshwater fishes of Madagascar: a study of endangered fauna with recommendations for a conservation strategy. Conservation Biology. 1991;5(2):231–243.

31. Bleeke P. Description de troutspecies inédites de Chromidoides de Madagascar. Verslagen Mededelingen der Koninklijke Akademie van Wetenschappen Amsterdam, Afdeeling Natuurkunde. 1868;2:307–314.

32. Stiassny MLJ, Sparks JS. Phylogeny and Taxonomic revision of the endemic Malagasy genus Psychochromis (Teleostei: Cichlidae), with the Description of five new species and a diagnosis for Katria, new genus. American Museum of Natural History. 2006;3535:1–55.

33. Sparks JS, Schaefer SA. Psychochromis inornatus: A new Cichlid (Teleostei: Cichlidae) from Northwestern Madagascar, with a discussion of intragenetic variation in Psychochromis.COPEIA. 2002;2002(1):120–130.

34. Loiselle PV. Fishes of the fresh waters of Nosy Be, Madagascar, with notes on their distribution and natural history. Ichtyological Exploration of Freshwaters. 2005;16(1):29–46.

35. Guinane S. Introducing the Tsipoy, Psychochromis nossibeensis. Cichlids News. 2000;9(2):28–31.

36. Martinez CM, Arroyave J, Sparks JS. A new species of Psychochromis from southeastern Madagascar (Teleostei: Cichlidae). Zootaxa. 2015;4044(1):79–92.

37. Loiselle PV. Captive breeding for the freshwater fishes of Madagascar. In: Goodman SM, Beansted JP, editors. The natural history of Madagascar. The University of Chicago Press, Chicago. 2003. p. 1569–1574.

38. Sparks JS, Reinthal PN. Paretroplus maromandia a new cichlid fish from the northwest of Madagascar. Occasional Papers. Museum of Zoology, University of Michigan. 1999;727:1–18.

39. Oguntanase BG. Distribution and Genetic Differentiation of the Populations of Flat-Headed Casimansace (Crossarchus platycephalus) in Nigeria. PhD Thesis, Federal University of Technology, Akure, Nigeria; 2017.

40. O佐Ostaz-Costaz A, Coutanceau JP, Bonillo C, et al. New insights into the chromosomal differentiation patterns among cichlids from Africa and Madagascar. Cytbium. 2017;41(1):35–43.

41. Loiselle PV. A review of the Malagasy Pachychanxus (Teleostei: Cyprinodontiformes, Aplochilidae) with descriptions of four new species. Zootaxa. 2006;1366(1):1–44.

42. Allgayer R. Description d’une nouvelle espèce du genre Paretroplus Bleeke (Teleostei: Cichlidae) de Madagascar. Revue française des Cichlidophiles. 1996;159:6–20.