

*Taxonomy and genetic relationships of Pangasiidae ..... (Rudhy Gustiano)*

## TAXONOMY AND GENETIC RELATIONSHIPS OF PANGASIIDAE, ASIAN CATFISHES, BASED ON MORPHOLOGICAL AND MOLECULAR ANALYSES

Rudhy Gustiano<sup>\*)</sup> and Laurent Pouyaud<sup>\*\*)</sup>

### ABSTRACT

Pangasiids are economically important riverine catfishes generally residing in freshwater from the Indian subcontinent to the Indonesian Archipelago. The systematics of this family are still poorly known. Consequently, lack of such basic information impedes the understanding of the biology of the Pangasiids and the study of their aquaculture potential as well as improvement of seed production and growth performance. The objectives of the present study are to clarify phylogeny of this family based on a biometric analysis and molecular evidence using 12S ribosomal mtDNA on the total of 1070 specimens. The study revealed that 28 species are recognised as valid in Pangasiidae. Four genera are also recognized as *Helicophagus* Bleeker 1858, *Pangasianodon* Chevey 1930, *Pteropangasius* Fowler 1937, and *Pangasius* Valenciennes 1840 instead of two as reported by previous workers. The phylogenetic analysis demonstrated the recognised genera, and genetic relationships among taxa. Overall, trees from the different analyses show similar topologies and confirm the hypothesis derived from geological history, palaeontology, and similar models in other taxa of fishes from the same area. The oldest genus may already have existed when the Asian mainland was still connected to the islands in the southern part about 20 million years ago.

**KEYWORDS:** taxonomy, genetic, morphology, molecular, catfish, pangasiidae

### INTRODUCTION

Pangasiidae are economically important riverine catfishes generally occurring in freshwater from the Indian subcontinent to the Indonesian Archipelago (Gustiano 2003; Gustiano and Pouyaud 2005; 2006). Morphologically, they are recognized by a laterally compressed body, two pairs of barbels, a short dorsal fin with two spines, a well developed adipose fin, a long anal fin, and a strong pectoral spine (Teugels 1996). The systematics of this family are still poorly known (Gustiano 2003; Pouyaud *et al.* 2005). Consequently, the lack of this basic information is a significant barrier to understanding the biology and hence the study of the aquaculture potential of Pangasiids, the improvement of their seed production and growth performance (Legendre 1999).

The objectives of the present study are to clarify phylogeny of this family based on a biometric analysis and molecular evidence using 12S ribosomal mtDNA on the total of 1070 specimens.

### MATERIALS AND METHODS

#### *Biometrics*

Nine hundred and ninety nine specimens were examined including the type of 49 previous described species housed in various museums. For each specimen, 35 point-to-point measurements covering the possible variations of the body were undertaken (Fig. 1).

Data were subjected to principal component analysis (PCA) (Bookstein *et al.* 1985). Measurements were log-transformed in order to minimise the effect of non-normality. The

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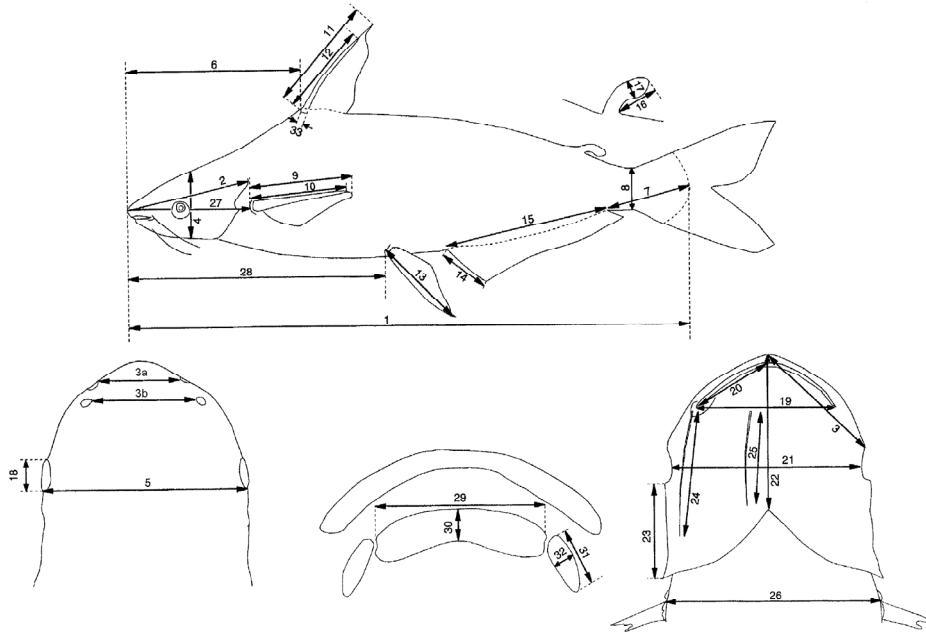


Figure 1. Measurements taken on *Pangasius* specimens: 1. Standard length; 2. Head length; 3. Snout length; 3a. Anterior snout width; 3b. Posterior snout width; 4. Head depth; 5. Head width; 6. Predorsal length; 7. Caudal peduncle length; 8. Caudal peduncle depth; 9. Pectoral fin length; 10. Pectoral spine length; 11. Dorsal fin length; 12. Dorsal spine width; 13. Pelvic fin length; 14. Anal fin height; 15. Anal fin length; 16. Adipose fin height; 17. Adipose fin width; 18. Eye diameter; 19. Mouth width; 20. Lower jaw length; 21. Interorbital length; 22. Distance snout to isthmush; 23. Postocular length; 24. Maxillary barbel length; 25. Mandibulary barbel length; 26. Body width; 27. Prepectoral length; 28. Prepelvic length; 29. Vomerine width; 30. Vomerine width; 31. Palatine length; 32. Palatine width; 33. dorsal spine width.

first factor, considered as the size factor, was not taken into account in order to minimise the size differences between samples. An independent PCA was run on the correlation matrix from untransformed data.

Finally, data analysis consisted of characterising groups from scatter plots between pairs of structuring characters for subsequent use in generic identification keys.

#### **Molecular analysis**

Fresh tissue samples were stored in liquid nitrogen for transfer to the laboratory. They were then stored at  $-20^{\circ}\text{C}$ . Mitochondrial analysis consisted of sequencing part of the 12S rDNA gene. One to five individuals were analysed for 28 species of Pangasiidae. One hundred mg of muscle were used to isolate total genomic DNA using a hexadecylmethyl-

ammoniumbromide extraction process (Doyle & Doyle, 1987). Primers used for the amplification of the mitochondrial gene included 12S light strand 5' - TTACACATGCAAGTCTCCGC - 3' and 12S heavy strand GTTACGACTTGCC TCCCCTT - 3' defined on the complete mitochondrial sequence of *Cyprinus carpio* (EMBL X61010, Chang *et al.* 1994).

DNA amplification and sequencing were performed following the procedures developed in Pouyaud *et al.* (2000). In order to check sequence accuracy and to correct any ambiguity bases, both strands were sequenced using each one of the two initial PCR primers.

Sequences of both strands were compared with each other and aligned using the sequence editor ESEE (version 3.1 s; Cabot & Beckenbach, 1989). Comparison of absolute numbers of transitions and tranversions were

calculated and plotted versus corrected genetic distances (using Kimura's distance two-parameter method, Kimura 1980). Phylogenetic inference was based on the neighbour joining method (Saitou & Nei 1987) (NEIGHBOR program in PHYLIP; Felsenstein 1993) from Kimura's distances among species. The reliability of the topologies was assessed with bootstrapping on 1000 replicates (SEQBOOT and CONSENSE programs in PHYLIP; Felsenstein 1993).

## RESULTS AND DISCUSSION

During the study twenty eight species were recognised as valid in Pangasiidae. Four genera were also recognized (*Helicophagus* Bleeker, 1858; *Pangasianodon* Chevey, 1930; *Pteropangasius* Fowler, 1937; and *Pangasius* Valenciennes, 1840) instead of two as reported by Vidhayanon (1993). Key below different genera is presented. For each genus, a key to the species is given.

### Key to genera

- 1a. 8-9 pelvic fin rays, long predorsal length (>37% SL), and slender dorsal spine width (3.5-5% HL).....***Pangasianodon***
- 1b. 6 pelvic fin rays .....2
- 2a. Slender anterior part of snout (<16.5% HL), posterior nostrils are in between anterior nostrils and orbit..... ***Helicophagus***
- 2b. Robust anterior part of snout (>16.5% HL), posterior nostrils close behind anterior ones and above imaginary line from anterior nostrils and orbit .....3
- 3a. Eye relatively large, minute maxillary barbel (<192% ED), dorsal and pectoral fins relatively thin, pectoral fin with minute and numerous serrations on the anterior and posterior edge of the fin, and minute adipose fin.....***Pteropangasius***
- 3b. Eye varies from small to large, relatively long maxillary barbel (>192% ED), dorsal and pectoral fins robust, and adipose fin relatively robust ..... ***Pangasius***

### Key to species of *Helicophagus*

- 1a. Anal rays 27-30; premaxillary teeth in a single curved band; gill rakers on the first branchial arch 27-33; eye diameter less than 9.3-13.5% HL; mandibular barbel less than 35%; anal fin length less than 32.9%; ..... ***Helicophagus typus***
- 1b. Anal ray counts more than 35; premaxillary teeth divided into two quadratic

- bands; gill rakers on the first branchial arch 7-18; eye diameter more than 14% HL; mandibular barbel more than 35%; anal fin length more than 33.6% ..... 2
- 2a. Vomerine tooth plate length 1.2-4.2% HL; vomerine tooth plate length is about one third of premaxillary tooth plate length ..... ***H. waandersii***
- 2b. Vomerine tooth plate length 5.1-8.9% HL; vomerine tooth plate length is about one of half premaxillary toothplate..... ***H. leptorhynchus***

### Key to species *Pangasianodon*

- 1a. Head length more than 30% SL; prepectoral length more than 26% SL; anterior part of snout width more than 32% HL; anal fin length less than 27.5% SL; distance between snout and isthmus less than 34% HL ..... ***Pangasianodon gigas***
- 2b. Head length less than 29% SL; prepectoral length less than 25% SL; anterior part of snout width less than 30% HL; anal fin length more than 27.5% SL; distance between snout and isthmus more than 37% HL .... ***Pangasianodon hypophthalmus***

### Key to species of *Pteropangasius*

- 1a. Predorsal length 30.8-34.4% SL; anal fin length 31-37.4% SL; anal fin rays 38-46; additional toothplates confluent with vomerine toothplate; abdomen with a well-developed median keel extending from throat to origin of anal fin ..... ***Pteropangasius pleurotaenia***
- 1b. Predorsal length 28.1-37.7% SL; anal fin length 22.8-35.2% SL; anal fin rays 26-40; additional toothplates of vomerine toothplate completely separated from vomerine toothplate ..... ***Pteropangasius micronemus***

### Key to species of *Pangasius*

- 1a. Vomerine toothplate without additional toothplate .....2
- 1b. Vomerine toothplate with additional toothplate .....5
- 2a. High adipose fin (5-6% SL) and narrow anterior part of snout width (22.7-27.2% HL).....***P. kinabatanganensis***
- 2b. Low adipose fin (less than 5% SL) and large anterior part of snout width (25.5-35.5% HL) .....3
- 3a. Anal fin length more than 31% SL and

- prepelvic length less than 44% SL ..... ***P. lithostoma***
- 3b. Anal fin length less than 31% SL and prepelvic length 42-52.9% SL ..... 4
- 4a. Dorsal spine width more than 7.7-9.3% HL and head width more than 14.1-15.6% SL ..... ***P. humeralis***
- 4b. Dorsal spine width 5.5-7.6% HL and head width 13.8-16.4% SL .... ***P. nieuwenhuisii***
- 5a. Filamentous fin rays ..... 6
- 5b. Non filamentous fin rays ..... 7
- 6a. Body width 21.1-23.2% SL, head length 23.2-30.2% SL, gill rakers on the first branchial arch 17-26..... ***P. sanitwongsei***
- 6b. Body width 16.9-21.9% SL, head length 20.3-28.3% SL, gill rakers on the first branchial arch 13-17..... ***P. larnaudii***
- 7a. Maxillary barbel length 100.5-203.9% HL, mandibular barbel 76.8-176.5% HL, and eye diameter 21.9-45% HL ..... ***P. acronym***
- 7b. Maxillary barbel less than 100.5% HL and mandibular less than 76.8% HL..... 8
- 8a. Predorsal length 25.1-31.2% SL and eye diameter 16.0-30.3% HL ..... ***P. polyuranodon***
- 8b. Predorsal length more than 30.1% SL ..... 9
- 9a. Long caudal peduncle (17.6-21.4% SL) and eye diameter 19.2-25.5% HL ..... ***P. elongatus***
- 9b. Short caudal peduncle (less than 17% SL) ..... 10
- 10a. Eye diameter 22.8-29.4% HL and predorsal length 30.1-32.7% SL ..... ***P. mahakamensis***
- 10b. Eye diameter less than 22.8% HL and predorsal length more than 31.8% SL ... 11
- 11a. Short distance snout isthmus (less than 110% SNL) ..... 12
- 11b. Long distance snout isthmus (more than 110% SNL) ..... 14
- 12a. Maxillary barbel length 79-97%; mandibular length 56-66% HL and dorsal spine width 4.4-5.7% HL..... ***P. sabahensis***
- 12b. Maxillary barbel length less than 80.7% HL, mandibular barbel length less than 52% HL; dorsal spine width more than 6.5% HL ..... 13
- 13a. Gill rakers on the first branchial arch 16-23 ..... ***P. mekongensis***
- 13b. Gill rakers on the first branchial arch 24-32 ..... ***P. kunyit***
- 14a. Predorsal length 31.8-35.3% SL, snout length 45.5-52.4% HL, anterior part of snout width 32.6-37.8% HL, posterior part of snout width 42.2-49.1% HL ..... ***P. krempfi***
- 14b. Predorsal length 34.6-42.8% SL, snout length 31.9-49.9% HL, anterior part of snout width 22.8-45.8% HL, and posterior part of snout width 26.8-39.5% HL ..... 15
- 15a. Dorsal spine width 4.7-6.2% HL, head length 19.6-23.2% SL, head width 11-14.2% SL, and body width 14.9-17% SL ..... ***P. rheophilus***
- 15b. Dorsal spine width 5.4-10.4% HL, head length 21.3-28.8% SL, head width 11.9-20.6% SL, body width 16.5-21.4% SL .... 16
- 16a. Higher gill rakers number on the first branchial arch (more than 27) ..... 17
- 16b. Lower gill raker number on the first branchial arch (less than 27) ..... 18
- 17a. 35-47 gill rakers on the first branchial arch, anterior part of snout width 26.4-31.1% HL.. ..... ***P. bocourti***
- 17b. 27-39 gill rakers on the first branchial arch, anterior part of snout width 29.3-36.6.5% HL ..... ***P. djambal***
- 18a. Predorsal length 33.5-37% SL and head length 21-24% SL ..... 19
- 18b. Predorsal length 36.1-42.8% SL and head length 23.8-28.8% SL ..... 20
- 19a. Dorsal spine width 8.1-8.9% HL and post ocular length 28.6-33.3% HL ..... ***P. myanmar***
- 19b. Dorsal spine width 6.3-7.7% HL and post ocular length 32.3-39.7% HL ..... ***P. pangasius***
- 20a. Width of mouth 31-41% HL, vomerine toothplate width 16-21.5% HL, and lower jaw length 17-24% HL ..... ***P. conchophilus***
- 20b. Width of mouth 41.9-52.5% HL, vomerine toothplate width 21.9-30.7% HL, and lower jaw length 23.9-31.5% HL ..... ***P. nasutus***

The phylogenetic analysis demonstrated the recognised genera, and the genetic relationships among the species (Fig. 2). Overall, trees from the osteological (Gustiano 2003), molecular and biometric analyses (Gustiano and Pouyau 2005) show similar topologies and confirm the hypothesis derived from geological history, palaeontology, and similar models in other taxa of fishes from the same area

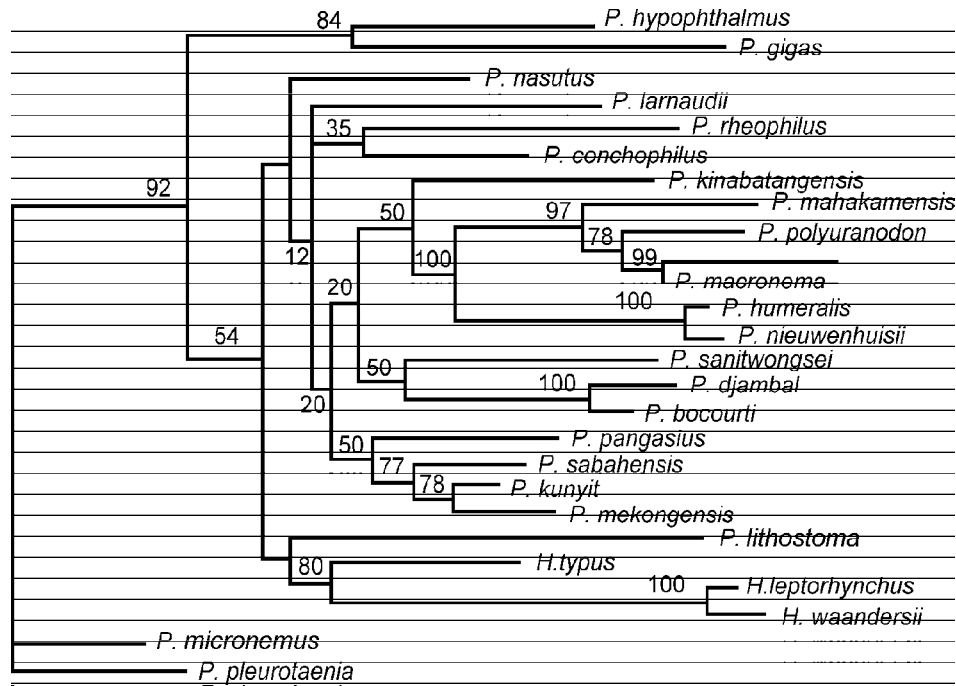


Figure 2. The nearest neighbor dendrogram for 12S rDNA gene within Pangasiidae. Numbers next to node show percentage group occurrence found with the 1000 bootstrapped trees.

(Gustiano 2003). The oldest genus may already have existed when the Asian mainland was still connected to the islands in the southern part about 20 million years ago (Gustiano *et al.* 2003; Pouyaud *et al.* 2005).

### CONCLUSION

The analysis demonstrated the genetic relationships among species of Pangasiids. The present study recognizes four genera i.e. *Helicophagus* Bleeker, 1858; *Pangasianodon* Chevey, 1930; *Pteropangasius* Fowler, 1937; and *Pangasius* Valenciennes, 1840. At this level, species belonging to the same genus are aggregated using the degree of similarity of morphometric and molecular data.

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### REFERENCES

- Bleeker, P. 1858a. Ichthyologiae Arcipelagi Indici Prodrum. Vol. I. Siluri. Lange & Co., Batavia, Nederland-Ind. 370 pp.
- Bookstein, F. L., B. Chernoff, R. Elder, J. Humphries, G. Smith and R. Strauss. 1985. Morphometrics in evolutionary biology. The geometry of size and shape change, with examples from fishes. *Acad. Nat. Sci., Philad., Spec. Publ.* 15: 1-277.
- Cabot, E.L. and A.T. Beckenbach. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Cabios* 5: 233-234.
- Chang Y.S., F.L. Huang and T.B. Lo. 1994. The complete nucleotide sequence and gene organization of Carp (*Cyprinus carpio*) mitochondrial genome. *J. Mol. Evol.* 38: 138-155.

- Chevey, P. 1930. Sur un nouveau silure géant du bassin du Mékong *Pangasianodon gigas* nov. g., nov. sp. *Bull. Soc. Zool. Fr.* 55: 536-542.
- Doyle J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.
- Felsenstein, J. 1993. *PHYLIP (Phylogeny Inference Package) Version 3.5c*. Seattle: University of Washington, USA.
- Fowler, H.E. 1937. Zoological results of the third de Schauensee Siamese Expedition, Part 8. Fishes obtained in 1936. *Proc. Acad. Nat. Sci. Philad.* 89: 125-264.
- Gustiano, R., G. Teugels, and L. Pouyaud. 2003. Revision of the *Pangasius kunyit* complex, with description two new species from South-East Asia (Siluriformes; Pangasiidae). *J. Nat. Hist.* 37: 357-376.
- Gustiano, R. 2003. Taxonomy and phylogeny of Pangasiidae catfishes from Asia (Ostariophysi, Siluriformes). Ph.D. Thesis, Leuven Univ., Belgium. 293 pp.
- Gustiano, R. and L. Pouyaud. 2005. Riverine catfishes of Kalimantan, Pangasiidae: Diagnosis, distribution and ecology. *Indon. Fish. Res. J.* 11: 59-66.
- Gustiano, R. and L. Pouyaud. 2005. Phenetic analysis of 28 species Pangasiid catfishes from Asia. *Zuriat* 16: 66-72.
- Gustiano, R. and L. Pouyaud. 2006. Diversity of pangasius catfishes from Sumatra. *Bul. Plasma Nutfah* 12: 83-88.
- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequence. *J. Mol. Evol.* 16: 111-120.
- Legendre, M. 1999. The catfish Asia project: Backgrounds, aims and prospects. In: The biological diversity and aquaculture of Clariid and Pangasiid Catfish in Southeast Asia. Proc. mid-term workshop of the "Catfish Asia project" (Editors: M. Legendre and A. Pariselle), IRD, Paris. p. 7-14.
- Pouyaud, L., G.G. Teugels, R. Gustiano and M. Legendre. 2000. Contribution to phylogeny of pangasiid catfishes based on allozymes and mitochondrial DNA. *J. Fish Biol.* 56: 1509-1538.
- Pouyaud, L., R. Gustiano and G. Teugels. 2004. Contribution to phylogeny of pangasiid catfishes based on mitochondrial 12S rDNA. *Indon. J. Agric. Sci.* 5: 45-72
- Teugels, G.G. 1996. Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi, Siluroidei): An overview. *Aquat. Living Resour.* 9: 9-34.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-525.
- Vidthayanon, C. 1993. Taxonomic revision of catfish family Pangasiidae. Ph.D. Thesis. Tokyo University of Fisheries. 203 pp.