INTRODUCTION

Pangasiids are economically important riverine catfishes generally occurring in fresh water 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

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
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°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 GTTACGACTTGCTCCTC-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 transversions 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 Vidthayanon (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 tooth plate........................*H. leptorhynchus*

Key to species Pangasianodon

1a. Head length more than 30%SL; protective 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
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<th>Species</th>
<th>Predorsal length</th>
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<th>Anterior part of snout width</th>
<th>Posterior part of snout width</th>
<th>Gill rakers on the first branchial arch</th>
<th>Dorsal spine width</th>
<th>Mandibular barbel length</th>
<th>Jaw length</th>
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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 Pouyaud 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.
Taxonomy and genetic relationships of Pangasiidae ....... (Rudhy Gustiano)

(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


