THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Genetics)

DEGREE

Genetics	Genetics
FIELD	**DEPARTMENT**

TITLE: Molecular Phylogenetic Relationships of Pangasiid and Schilbeid Catfishes in Thailand

NAME: Miss Wirangrong Karinthanyakit

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Professor Amnuay Jondeung, Dr.Phil.)

COMMITTEE MEMBER

(Associate Professor Panapa Saksoong, Ph.D.)

COMMITTEE MEMBER

(Assistant Professor Arunee Engkagul, Ph.D.)

DEPARTMENT HEAD

(Associate Professor Somsak Apisitwanich, Dr.Agr.Sci.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

MOLECULAR PHYLOGENETIC RELATIONSHIPS OF PANGASIID AND SCHILBEID CATFISHES IN THAILAND

WIRANGRONG KARINTHANYAKIT

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Genetics)
Graduate School, Kasetsart University
2012

Wirangrong Karinthanyakit 2012: Molecular Phylogenetic Relationships of Pangasiid and Schilbeid Catfishes in Thailand. Doctor of Philosophy (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Professor Amnuay Jondeung, Dr.Phil. 158 pages.

The pangasiid and schilbeid catfishes are two economically important families in Thailand. The taxonomy and phylogeny of the both is rather problematic and there is still debate on the number of pangasiid genera, their intergeneric relationships and the relative position of the enigmatic schilbeid species, *Clupisoma sinense*. In order to resolve problematic classification of pangasiids and schilbeids and to obtain the robust phylogeny, phylogenetic relationships among 11 pangasiids and 4 schilbeids of Thailand along with 2 pangasiids and 2 schilbeids from Sumatra were reconstructed based on mitochondrial *cytochrome b*, *12S rRNA*, $tRNA^{Val}$ and *16S rRNA* as well as partial nuclear *RAG1* gene sequences using neighbor-joining, maximum parsimony, maximum likelihood and Bayesian inference methods. The phylogenies recovered Pangasiidae and Schilbeidae as monophyletic groups. The four genera of Pangasiidae: *Pangasius*, *Pseudolais*, *Helicophagus* and *Pangasianodon* were grouped into three major clades as *Pangasius*, *Pseudolais* + *Helicophagus* and *Pangasianodon*. *Pangasianodon* was supported as the most basal taxon, whereas *Pseudolais* + *Helicophagus* were recovered as sister group of *Pangasius*. Within Schilbeidae, three main clades were recovered as *Laides* + *Clupisoma sinense*, *C. prateri* + *Eutropiichthys* and *Pseudeutropius* which was recognized as the most basal lineage. *C. sinense* was grouped rather to *L. longibarbis* than to *C. prateri*. On the basis of phylogenetic analyses and sequence divergences, *C. sinense* should be categorized as *L. sinensis*. Pangasiidae and Schilbeidae have diverged from a common ancestor probably in Miocene period. The dispersal of pangasiids occurred in the late Miocene to the late Pleistocene, while the emergence of schilbeids initiated in the middle Miocene to the middle Pliocene which was more ancient than pangasiids.

_____ _____ ___ / ___ / ___

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to gratefully thank Prof. Dr. Amnuay Jondeung for his valuable suggestions and supports throughout the duration of my study and Dr. Rafael Zardoya at the Museo Nacional de Ciencias Naturales, CSIC in Madrid, Spain for providing me invaluable advice in the laboratory as well as phylogenetic knowledge during my stay in Spain. I would like sincerely to thank Assoc. Prof. Dr. Panapa Saksoong and Asst. Prof. Dr. Arunee Engkagul my committees for their valuable comments and suggestions.

I would like to sincerely thank Dr. Tan Heok Hui, Mr. Chaiwut Grudpan and Mrs. Jarungjit Grudpan for kindly providing some specimens used in this research. I am heartfelt thank to all my colleagues in Department of Genetics, Kasetsart University and also my colleagues in the laboratory of Dr. Rafael Zardoya for their kind helps.

This research could not be completed if there were no finantial support provided by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0228/2546). I am extremely grateful this program to give me not only a grant, but also the great opportunity for the education in genetics study at Kasetsart University.

I am especially appreciated my parents and my brother for their unconditional support and encouragements. Finally, I deeply appreciated Mr. Santi Wongsan who always devoted time, support and will power my success. This thesis and everything else I will ever do, will always be dedicated to them.

Wirangrong Karinthanyakit

October 2012

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

ATPase 6-8	=	*ATP synthase subunits 6* and *8*
BLASTn	=	Basic Local Alignment Search Tool-nucleotide
BI	=	Bayesian inference
bp	=	base pair
ºC	=	degree Celsius
COI	=	*cytochrome oxidase 1*
Cyt b	=	*cytochrome b*
DNA	=	deoxyribonucleic acid
dNTP	=	deoxynucleotide triphosphate
EtBr	=	ethidium bromide
g	=	gram
GTR	=	General Time Reversible model
HKY	=	Hasegawa, Kishino and Yano model
ILD	=	Incongruence Length Difference test
IUCN	=	the World Conservation Union
Kb	=	kilobase
min	=	minute
ML	=	maximum likelihood
ml	=	milliliter
MP	=	maximum parsimony
mtDNA	=	mitochondrial DNA
ND1-2	=	*NADH dehydrogenase subunits 1* and *2*
ng	=	nanogram
µg	=	microgram
µl	=	microliter
NJ	=	Neighbor-joining
PCR	=	Polymerase Chain Reaction
RNA	=	ribonucleic acid
rRNA	=	ribosomal ribonucleic acid
12S rRNA	=	small subunit of ribosomal RNA

LIST OF ABBREVIATIONS (Continued)

16S rRNA	=	large subunit of ribosomal RNA
tRNA	=	transfer ribonucleic acid
RAG 1	=	*Recombination Activating Gene 1*
TAE	=	Tris-Acetate-EDTA electrophoresis buffer solution
TE	=	buffer solution containing Tris and EDTA
UV	=	ultra violet
Val	=	valine

MOLECULAR PHYLOGENETIC RELATIONSHIPS OF PANGASIID AND SCHILBEID CATFISHES IN THAILAND

INTRODUCTION

Siluriformes is a very diverse group of bony fish, with approximately 416 genera and over 2500 species (Diogo, 2003). Pangasiidae and Schilbeidae are riverine catfishes and distribute in the large rivers throughout southern Asia and Sunda Shelf. Thailand has more pangasiids than any other countries with about 17 recognized species in both families (Vidthayanon and Roongthongbaisuree, 1993, Ferraris, 2007) and they distribute mainly in three rivers, the Chao Phraya, the Mekong and the Salween which constitute together the most species rich area within the Indochinese region (Vidthayanon, 1993). Fishes in the wild have been over-exploited for a long time, therefore, some species become critically endangerd species such as *Pangasianodon gigas* and *Pangasius sanitwongsei* in IUCN Red List (IUCN, 2010). Many species such as *P. gigas*, *P. hypophthalmus*, *Pangasius larnaudii*, *Laides longibarbis a*nd *Clupisoma sinense* are the important food sources and are raising now in aquaculture.

The members of the families Pangasiidae and Schilbeidae exhibit a rather similar morphology that confounded ichthyologists in the past, and in fact, some pangasiid species, such as *Pangasius polyuranodon*, *P. sanitwongsei*, and *Pangasianodon gigas* were previously included in the family Schilbeidae based on the number of barbels, vomerine and palatine teeth, nostril position and abdomen characteristics (Smith, 1945), whereas the schilbeid genus *Laides* was formerly identified as belonging to the family Pangasiidae (Roberts and Vidthayanon, 1991). The initial classification of Pangasiidae and Schilbeidae as distinct families was based on differences in the anatomy of the Weberian apparatus (Nelson, 1976 and Burgess, 1989). This hypothesis was widely accepted by subsequent authors, who found additional synapomorphies to support the monophyly of each family (Mo, 1991; de Pinna, 1998; Diogo, 2003).

Based on morphological approaches, there is no agreement on the number of genera within Pangasiidae as yet. Some studies described up to four genera namely *Pangasius*, *Helicophagus*, *Pseudolais* (or *Pteropangasius*) and *Pangasianodon* (Vidthayanon and Roongthongbaisuree, 1993; Ferraris, 2007), whereas others classified pangasiids only into two genera i.e., *Pangasius* and *Helicophagus* (Roberts and Vidthayanon, 1991; Vidthayanon, 1993; Pouyaud *et al*., 1999; Teugels, 2003; Gustiano *et al*., 2004). Another study (Rainboth, 1996) questioned the status of *Pseudolais* as an independent genus, and considered it a subgenus of *Pangasius*. Thus three genera of Pangasiidae including *Helicophagus, Pangasius* and *Pangasianodon* were recognized. The taxonomy of the family Schilbeidae is also dubious as some studies (de Pinna, 1998; Diogo *et al*., 2004) supported the monophyly of the group, whereas others (Howes, 1985; Mo, 1991; Teugel, 1996) questioned it. Moreover, phylogenetic intrarelationships of the Schilbeidae await a detailed phylogenetic study. For instance, the enigmatic *Clupisoma sinense* has been placed in the genus *Laides* (Teugels, 2003).

Given that morphology has rendered rather ambiguous classifications within pangasiids and schilbeids, molecular data may be helpful in resolving their phylogenetic intrarelationships. Mitochondrial (mt) DNA has undoubtedly become the most widely used tool for animal molecular phylogenetic studies nowadays due to its features: for instance, absence of introns, maternal inheritance, practical absence of recombination and haploidy (Meyer, 1993; Avise, 1994). Of the various mt genes, *cytochrome b* (*cyt b*) has proven to be a robust evolutionary marker for the determination of the phylogenies at various taxonomic levels in fishes (Meyer, 1994; Johns and Avise, 1998; Peng *et al*., 2004; Rüber *et al*., 2004; Sloss *et al*., 2004; Doiuchi and Nakabo, 2006; Perdices *et al*., 2008; Šlechtová *et al*., 2008). Since the ribosomal genes (*12S* and *16S rRNA*) evolve at a slower rate than mt protein coding genes, they have been successfully used for higher taxonomic level in phylogenetic analyses (Hillis and Dixon, 1991; Wiley *et al*., 1998). Numerous studies have used *12S*/*16S rRNA* sequences to resolve evolutionary relationships at familial level of fishes (Orrell and Carpenter, 2004; Shimabukuro-Dias *et al*., 2004; Sloss *et al*., 2004; Doiuchi and Nakabo, 2006). To provide independent data from a different genome,

several recent studies have utilized some orthologous nuclear protein coding genes for the inference of phylogenetic relationships. One of the most widely applied nuclear protein coding genes is the *Recombination Activating Gene 1* (*RAG1*). This gene possesses various properties such as a highly stationary base composition, scarcity of indels, and a minimal saturation of transition changes at the third codon positions render it ideal for the phylogenetic reconstruction in general (Groth and Barrowclough, 1999; Martin, 1999).

Thus far, very few attempts were conducted to infer the phylogenetic relationships of the families Pangasiidae and Schilbeidae using molecular data. Within Pangasiidae, the first molecular phylogenetic hypothesis of Pangasiidae was proposed based on the analysis of allozyme data (Pouyaud *et al*., 1998). By Fitch cluster analysis, four genetic differentiated groups were recognized as *Helicophagus*, *Pangasianodon*+*Pteropangasius*, *Pangasius* 1 and *Pangasius* 2. Among these groups, *Pangasianodon* and *Pangasius* were recovered as polyphyletic. The second study attempted to resolve pangasiid phylogenetic relationships based on a small fragment (539 bp) of the mt *cyt b* gene and allozyme data (Pouyaud *et al*. 2000) and the third study based on partial sequences of the mt *12S rRNA* gene (Pouyaud *et al*. 2004). Although the results from these two studies recovered four possible pangasiid genera, however, the interrelationships among them were either unresolved or incongruent. Pouyaud *et al*. (2000) proposed *Helicophagus* and *Pteropangasius* as the basal group of Pangasiidae and *Pangasius* as well as *Pangasianodon* as more derived lineage. In contrast, Pouyaud *et al*. (2004) proposed *Pteropangasius* as the most basal taxon within Pangasiidae and *Pangasianodon* as a sister group to *Pangasius* and *Helicophagus*. The lack of resolution and/or incongruence of recovered pangasiid trees in previously molecular studies may be likely related with insufficient phylogenetic informative characters (Crow *et al*., 2004). Within Schilbeidae, Pouyaud *et al*. (2004) proposed the *12S rRNA* phylogeny of seven species of Asian and African schilbeids. NJ tree demonstrated that the Schilbeidae seems to be a monophyletic group, but with low statistical support with the relationships as (*Schilbe*, (*Pseudeutropius*, ((*Clupisoma*, *Laides*), (*Eutropiichthys*, *Silonia*)))). *L. sinensis* was aggregated with *L. hexanema* and this is in contrast with

the presently classification in which *L. sinensis* was placed to the genus *Clupisoma* as *C. sinense*. Thus, question still remain about the validity of this species and its phylogenetic position should be determined with the extensive molecular data.

In recent years, several new species of pangasiids and schilbeids including *Helicophagus leptorynchus* (Ng and Kottelat, 2000), *Laides longibarbis* (Ng, 1999) and *Eutropiichthys salweenensis* (Ferraris and Vari, 2007) have been reported based on morphological evidences. However, there is no molecular evidence to pinpoint the taxonomic position of these recently recognized species, thus the validity of these species will also be assessed and discussed here.

In this study, the molecular phylogenies of Thai pangasiid and schilbeid species were deduced from information of the multiple loci including the mt *cyt b*, the contiguous fragment of the posterior half of the *12S rRNA*, $tRNA^{Val}$ and the anterior half of the *16S rRNA* gene (hereafter referred to as RNA data set) as well as the nuclear *RAG1* gene. The recovered phylogenies were used to discern competing hypotheses on the intra- and intergeneric relationships within members of Thai pangasiids and schilbeids, and to clarify the problematic taxonomy of these two families. Phylogenetic analyses clearly recognized two major clades as Pangasiidae and Schilbeidae. Four genetic differentiated lineages of Pangasiidae corresponding to the genera *Pangasius*, *Helicophagus*, *Pseudolais* and *Pangasianodon* were recovered with the placement of *Pangasianodon* as basal group. Three main clades were recognized within Schilbeidae as *Laides* + *Clupisoma sinense*, *C. prateri* + *Eutropiichthys* and *Pseudeutropius* which was the most basal lineage. *C. sinense* was closely related to *L. longibarbis* rather than to *C. prateri* and a recategorization of *C. sinense* to the genus *Laides* is suggested. Phylogenetic approach in combination with sequence divergences validated the species status of *L. longbarbis* and *E. salweenensis* but suggests that *H. leptorhynchus* might be considered as the synonym of *H. waandersii*. Phylogenetic-based fossil calibrated analysis found that the dispersal within Pangasiidae occurred in the late Miocene to the late Pleistocene, while the isolation within Schilbeidae initiated at the middle Miocence and extended to the middle Pliocene.

OBJECTIVES

The main objective of this work was to clarify the taxonomic status of individual members of the families Pangasiidae and Schilbeidae in Thailand employing mitochondrial and nuclear DNA sequences. Thus, three specific objectives were as follows:

1. To draw intra- and intergeneric relationships of the families Pangasiidae and Schilbeidae.

2. To determine the number of genera within Pangasiidae, the generic position of enigmatic schilbeid species, *Clupisoma sinense* and to validate the three putative new species status of *Helicophagus leptorhynchus*, *Laides longibarbis* and *Eutropiichthys salweenensis*.

3. To estimate the divergence times between Pangasiidae and Schilbeidae, and within both families.

LITERATURE REVIEW

Pangasiidae and Schilbeidae are morphologically closely related families originated from the same Indian subcontinent and then distributed to southern Asia (Vidthayanon and Roongthongbaisuree, 1993). The family Pangasiidae consists of 30 valid species and confines only to southern and Southeast Asia (Ferraris, 2007). Thailand is also a home of these catfishes. Pangasiids are more prominent in Thailand than in any other countries else and consist of four extant genera with 11 valid species (Ferraris, 2007). The family Schilbeidae contains 15 genera with 64 species (Ferraris, 2007) which distributes in fresh-water bodies of Africa and southern Asia (Teugels, 2003). Even though ten genera of schilbeids are known to be native to Asia, but only four genera with five species (four extant and one extinct species, *Platytropius siamensis*) are found in Thailand (Diogo *et al*., 2004). Members of Thai pangasiids and schilbeids are mainly found in three major rivers including the Chao Phraya, Mekong and Salween Rivers which are the most speciose area within the Indochinese region (Vidthayanon, 1993).

1. Taxonomic background of Pangasiidae and Schilbeidae

Under current taxonomic classification in regarding to Nelson (1994), Pangasiidae and Schilbeidae belong to Phylum Chordata; Class Actinopterygii; Subclass Neopterygii; Division Teleostei; Order Siluriformes.

A. Family Pangasiidae

The family Pangasiidae is characterized by a laterally compressed body, a short dorsal fin with one or two spines, a well-developed but small adipose fin, a long anal fin with 26-46 rays, pelvic fin with 6 or 8-9 rays, strong pectoral spines and two pairs of barbels (maxillary and mandibular) (Nelson, 1994). Pangasiid species exhibit great differences in their body size e.g. *Pangasianodon gigas* and *Pangasius sanitwongsei* are two species which can reach up to 3 m (Roberts and Vidthayanon, 1991). Medium sized species achieve a maximum length of around 80-100 cm such

as *Pangasius bocourti* and *P. larnaudii*. The small ones can vary between 20 and 40 cm such as *Pangasius macronema* and *Pseudolais pleurotaenia* (Roberts and Vidthayanon, 1991). Based on the recent checklist of catfishes, Pangasiidae contains five genera with 30 species (Ferraris, 2007). The description for pangasiid genera and species is given below and their distribution is shown in Figure 1.

1. *Cetopangasius*

The *Cetopangasius* is the fossil genus which contains only single species *C. chaetobranchus.* Its fossil in age of middle or late Miocene was firstly discovered at Ban Nong Pla in Phetchabun Province, north-central Thailand (Roberts and Jumnongthai, 1999). This new species is distinguished from all other living pangasiids by its extremely elongate and numerous gill rakers, low counts of vertebrae (especially of abdominal vertebrae, 14-15) (vs. 15-23) and high counts of anal fin rays (38-42 vs. 25-44) (Roberts and Jumnongthai, 1999).

2. *Helicophagus*

The molluscivorous genus *Helicophagus* differs from other pangasiids in having a much narrower mouth and snout and the absence of palatine tooth patches (Pouyaud *et al*., 1999). This genus comprises three species, *H.waandersii* from Sumatra and Peninsular Malaysia (Ng and Kottelat, 1999), *H. typus* from Sumatra and Borneo (Roberts and Vidthayanon, 1991) and *H. leptorhynchus* from the Chao Phraya and Mekong Rivers in Indochina (Ng and Kottelat, 1999). *H. typus* has marked differences from its two congeners in shape of palatal toothbands (premaxillary teeth in a single curved patch vs. premaxillary teeth in two quadratic patches), numbers of gill rakers (27-10 vs. 8-12) and anal fin rays (30-31 vs. 38-42) (Vidthayanon, 1993). *H. waandersii* and *H. leptorhynchus* are morphologically similar species but they are different in length of anal fin, caudal peduncle, head and eye diameter (Ng and Kottelat, 2000).

3. *Pangasianodon*

Pangasianodon is diagnosed by the possession of 8-9 pelvic fin rays (instead of only 6 in others) and a terminal mouth, with teeth of upper jaw entirely covered by lower jaw when mouth is closed (Roberts and Vidthayanon, 1991). It contains two species, *P. gigas* from the Mekong River basins and *P. hypophthalmus* from the Mekong and Chao Phraya River basin (Ferraris, 2007). *P. gigas* can be differentiated from *P. hypophthalmus* in having very small, rudimentary or absent gill rakers (vs. normally developed gill rakers) and swimbladder confined to abdomen (vs. extended beyond abdominal to the base of anal fin) (Roberts and Vidthayanon, 1991; Vidthayanon, 1993).

4. *Pangasius*

The genus *Pangasius*, with 22 valid species (Ferraris, 2007) exhibits great morphological and ecological diversity (Pouyaud *et al*., 2000). All *pangasius* species share an elastic spring formation of the parapophysis of the fourth Weberian vertebra, which is not sutured to the posttemporal (Vidthayanon, 1993). A single fossil species, *P. indicus* was described from Central Sumatra, however, the reported age from the Eocene is debatable (Ferraris, 2007). On the basis of fin, swimbladder chamber and pelvic girdle characters, *Pangasius* species can be divided into four subgroups (Vidthayanon, 1993):

The first subgroup contains *P. larnaudii* and *P. sanitwongsei* which possess the filamentous or elongated tips of dorsal, pectoral and pelvic fins, median lamina of the pelvic girdle, a two-chambered swimbladder and spatula-like nasal bone. *P. larnaudii* differs from *P. sanitwongsei* in having a large, black humeral spot. Both species only distribute in the Mekong and Chao Phraya River basins.

The second subgroup which comprises six species, *P. pangasius*, *P. myanmar*, *P. conchophilus*, *P. nasutus*, *P. bocourti* and *P. djambal* shares the same aforementioned characters with *P. larnaudii* and *P. sanitwongsei*, except for the

absence of fin elongation and rod-like nasal bone. *P. pangasius* is only found in Indian subcontinent. Its posterior lobe of swimbladder extends to base of anal fin. *P. myanmar* found only in Myanmar possesses 32 anal fin rays, 20-21 gill rakers and 46-47 vertebrae. *P. conchophilus* is similar to *P. myanmar* but has 25-30 anal fin rays, 15-19 gill rakers and 39-44 vertebrae which are fewer than those found in *P. myanmar*. It is known from Mekong, Bangpakong, and Chao Phraya River basins. *P. nasutus* from Sumatra, Borneo and Malay Peninsula is characterized by the possession of strongly projected snout and vomerine toothpatch exposed entirely when mouth closed. *P. bocourti* from Mekong and Chao Phraya River basins has marked differences from the others in numerous gill rakers (35-48) and *P. djambal* from Java and Borneo has 24-35 gill rakers.

The third subgroup contains three species, *P. macronema*, *P. krempfi* and *P. polyuranodon* which share the characteristics of a three-chambered swimbladder and no median lamina of pelvic girdle. *P. macronema* is characterized by the presence of abdominal stripes and gill rakers more than 37. It is known from the Mekong and Chao Phraya Rivers, Java, and Borneo (Kottelat, 2001). *P. krempfi* has a relatively elongated snout and two crescentic patches of palatal teeth. It is found in the Mekong River and along the coast of South China Sea of Vietnam and Guandong, China (Kottelat, 2001). *P. polyuranodon* differs from the others in having a large median vomerine tooth patch, very small palatine tooth patch and maxillary barbels extending posteriorly to gill opening and it distributes in the Chao Phraya and Mekong Rivers and rivers of Sumatra and Borneo (Pouyaud *et al*., 2002).

The last subgroup which comprises four species, *P. humeralis*, *P. kinabatanganensis*, *P. lithostoma* and *P. nieuwenhuisii* shares the characters of a single, enlarged vomerine toothband without lateral extensions and a two-chambered swimbladder. *P. humeralis* from western Borneo is most similar to *P. nieuwenhuisii* from eastern Borneo but differs from *P. nieuwenhuisii* in having a black pectoral fin (vs. dusky or plain pectoral fin) and rounded snout (vs. pointed snout) (Vidthayanon and Roongthongbaisuree, 1991). *P. kinabatanganensis* from northeastern Borneo differs from all other *Pangasius* except *P. lithostoma* from western Borneo in having

a relatively flat palatal tooth patch, not projecting strongly down from roof of mouth. It differs from *P. lithostoma* in having only 27-30 anal fin rays (instead of 40-41) (Vidthayanon and Roongthongbaisuree, 1991).

Besides those subgroups, another six *Pangasius* species, *P. elongatus*, *P. kunyit*, *P. mahakamensis*, *P. mekongensis*, *P. rheophilus* and *P. sabahensis* have been recently described as new species. *P. elongatus* from Vietnam is characterized by an elongated body with a moderate predorsal length, a short snout length, a long caudal peduncle, short mandibular barbels and large eyes (Pouyaud *et al*., 2002). *P. mahakamensis* from the Mahakam River is recognized from the other species of *Pangasius* by the short caudal peduncle, large eye, short mandibulary barbel and short predorsal length (Pouyaud *et al*., 2002). *P. kunyit* which is restricted to Sumatra and Kalimantan differs from other congeners by a higher number of gill rakers on the first branchial arch (24-32) (Gustiano *et al*., 2003). *P. mekongensis* from the lower Mekong River (Vietnam) is characterized by the combination of a long, broad and rounded head with an elongated snout, the short distance from the snout to the isthmus, a robust dorsal spine, the short palatine toothplates and the possession of 16-23 gill rakers. *P. sabahensis* from the Kinabatangan River Basin (Malaysia) is morphologically closely related to *P. mekongensis* but possesses the longer mandibular and maxillary (Gustiano *et al*., 2003). *P. rheophilus* which is described from Indonesia can be differentiated from other congeners in having a large vomerine tooth plate bordered by long and slender palatine tooth plates (Pouyaud and Teugels, 2000).

5. *Pseudolais*

The genus *Pseudolais* (Ferraris, 2007) or *Pteropangasius* (Roberts and Vidthayanon, 1991; Vidthayanon, 1993; Vidthayanon and Roongthongbaisuree, 1993) is characterized by the possession of posteriormost chamber of swimbladder segmented into many small chambers and the parapophysis of the fourth Weberian vertebra sutured with the posttemporal (Vidthayanon, 1993). It contains only two species, *P. micronemus* from the Mekong and Hue Rivers, Malay Peninsula, Sumatra,

Java, and Borneo (Roberts & Vidthayanon, 1991) and *P. pleurotaenia* from the Mekong, Meklong, Tapi and Chao Phraya River basins (Kottelat, 2001). *P. pleurotaenia* differs from *P. micronemus* in having an entirely keeled of abdomen and 39-46 anal fin rays (vs. abdomen rounded anterior to pelvic fins and 28-32 anal fin rays) (Roberts and Vidthayanon, 1991).

Of all 28 living pangasiid species which distribute throughout southern and Southeast Asia, 11 species have been described from Thailand (Roberts and Vidthayanon, 1991; Ferraris, 2007) as follows: *H. leptorhynchus*, *P. gigas*, *P. hypophthalmus*, *P. bocourti*, *P. conchophilus*, *P. krempfi*, *P. larnaudii*, *P. macronema*, *P. polyuranodon*, *P. sanitwongsei*, and *P. pleurotaenia*. *H. leptorhynchus* has been recently recognized as a valid species from the Chao Phraya and Mekong Rivers (Ng and Kottelat, 2000). It is morphologically similar to that has been previously identified as *H. waandersii* (Roberts and Vidthayanon, 1991; Vidthayanon and Roongthongbaisuree, 1993), but differs from it in having a longer anal fin, shorter caudal peduncle, longer head and larger eye. *H. leptorhynchus* also has a more slender snout (when viewed laterally) than that of *H. waandersii.*

Based on morphological and anatomical characteristics, the classification of the family Pangasiidae has been uncertain. This is illustrated by the unstable generic classifications of extant pangasiids which have been proposed by several authors and the number of recognized genera varied from two to four (Table 1). Roberts and Vidthayanon (1991) revised the family Pangasiidae by considering the important morphological characteristics such as shape of head, oral and abdomen, number of gill rakers, fin rays, palatal toothbands and color, and proposed only two pangasiid genera, *Pangasius* and *Helicophagus*. Later, Vidthayanon (1993) presented more clearly elucidate identification and classification of the family Pangasiidae through examination of the external characteristics (e.g. head shape, eye and mouth position), morphometric measurement, meristic counts such as head and body length, number of gill rakers, fin rays, and vertebrae as well as anatomical studies. He agreed with Roberts and Vidthayanon (1991) that classified pangasiids into two genera, *Pangasius* and *Helicophagus*. In the same year, based on the morphological

characteristics including Weberian vertebrae, head shape, barbels, nostrils, mouth and dentition, swimbladders, fins and color patterns, Vidthayanon and Roongthongbaisuree (1993) elevated the *Pteropangasius* (or *Pseudolais*; Ferraris, 2007) and *Pangasianodon* and recognized four pangasiid genera, *Pangasius*, *Helicophagus*, *Pteropangasius* and *Pangasianodon*. However, based on the features of barbels, palatal teeth, pelvic fin rays and the position of anterior and posterior nostril, Rainboth (1996) synonymized *Pteropangasius* with *Pangasius*, therefore Pangasiidae has been classified into *Helicophagus, Pangasius* and *Pangasianodon*. Later, several authors (Pouyaud *et al*., 1999; Teugels, 2003; Gustiano *et al*., 2004) encouraged the generic classification within pangasiids into only two genera, *Pangasius* and *Helicophagus* and proposed four subgenera of *Pangasius* including *Neopangasius*, *Pteropangasius*, *Pangasianodon* and *Pangasius*. Recently, Ferraris (2007) in his report "Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes)" proposed five pangasiid genera consisting one extinct genus, *Cetopangasius* and four extant genera, *Pangasius*, *Helicophagus*, *Pseudolais* (or *Pteropangasius*) and *Pangasianodon.*

As mentioned above, based on morphological characteristics, it is unclear how many genera in the family Pangasiidae. Vidthayanon and Roongthongbaisuree (1993) and Ferraris (2007) recognized four extant genera including *Pangasius*, *Helicophagus*, *Pteropangasius* (or *Pseudolais*) and *Pangasianodon*. Rainboth (1996) suggested three pangasiid genera; *Pangasius*, *Helicophagus* and *Pangasianodon*, with the synonymization of *Pteropangasius* with *Pangasius*. Several studies (Roberts and Vidthayanon, 1991; Vidthayanon, 1993; Pouyaud *et al*., 1999; Teugels, 2003; Gustiano *et al*., 2004) recognized only two genera, *Pangasius* and *Helicophagus*, with the synonymization of *Pteropangasius* and *Pangasianodon* with *Pangasius* (Roberts and Vidthayanon, 1991) or elevated *Pteropangasius* and *Pangasianodon* to subgenera of the genus *Pangasius* (Vidthayanon, 1993; Pouyaud *et al*., 1999; Teugels, 2003; Gustiano *et al*., 2004).

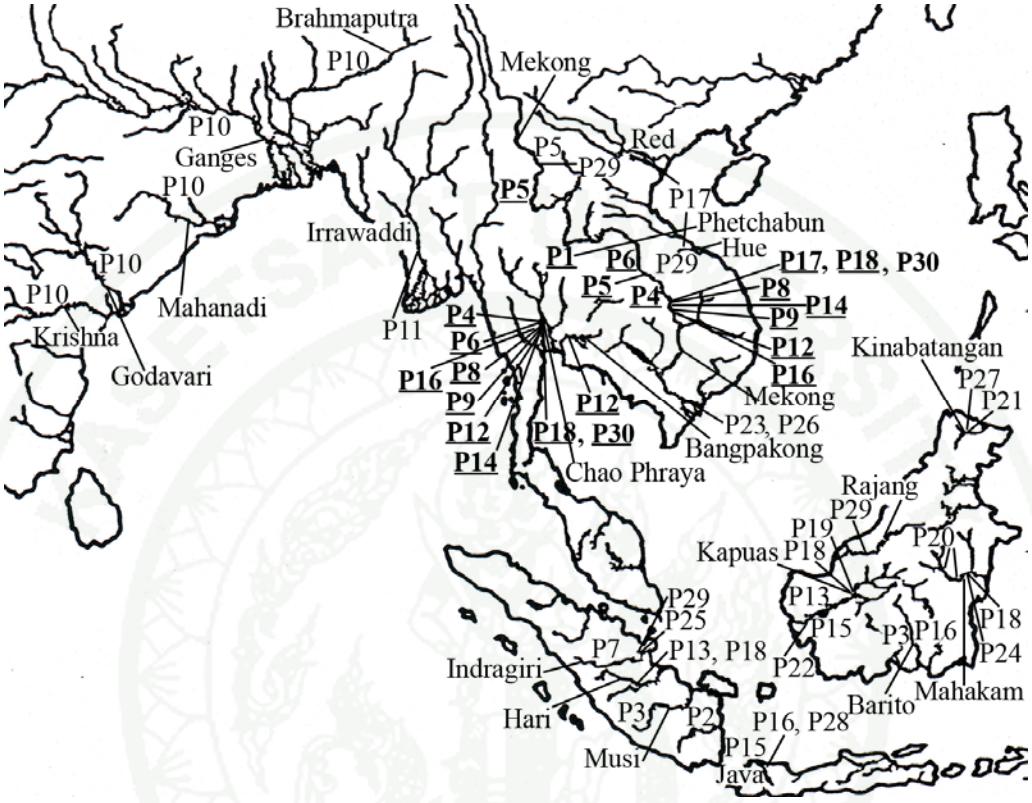


Figure 1 River map of southern and Southeast Asia showing the distribution of the Pangasiidae.

P1-P30 is assigned for each species: P1 = *Cetopangasius chaetobranchus*, P2 = *Helicophagus waandersii*, P3 = *H. typus*, P4 = *H. leptorhynchus*, P5 = *Pangasianodon gigas*, P6 = *P. hypophthalmus*, P7 = *Pangasius indicus*, P8 = *P. larnaudii*, P9 = *P. sanitwongsei*, P10 = *P. pangasius*, P11 = *P. myanmar*, P12 = *P. conchophilus*, P13 = *P. nasutus*, P14 = *P. bocourti*, P15 = *P. djambal*, P16 = *P. macronema*, P17 = *P. krempfi*, P18 = *P. polyuranodon*, P19 = *P. humeralis*, P20 = *P. nieuwenhuisii*, P21 = *P. kinabatanganensis*, P22 = *P. lithostoma*, P23 = *P. elongatus*, P24 = *P. mahakamensis*, P25 = *P. kunyit*, P26 = *P. mekongensis*, P27 = *P. sabahensis*, P28 = *P. rheophilus*, P29 = *Pseudolais micronemus*, P30 = *P. pleurotaenia*. P1, P4-P6, P8-P9, P12, P14, P16-P18 and P30 in bold font (with underline) represent pangasiid species in Thailand.

Table 1 Generic classification within the Pangasiidae previously proposed in the literatures based on morphological data.

Robert and Vidthayanon (1991); Vidthayanon (1993); Pouyaud *et al*. (1999), Teugels (2003); Gustiano *et al*. (2004)	Vidthayanon and Roongthongbaisuree (1993); Ferraris (2007	Rainboth (1996)
Helicophagus *Pangasius*	*Helicophagus* *Pangasius* *Pteropangasius* (or *Pseudolais*; Ferraris, 2007) *Pangasianodon*	*Helicophagus* *Pangasius* *Pangasianodon*

B. Family Schilbeidae

The family Schilbeidae is characterized by a laterally compressed body; usually four pairs of barbels; dorsal fin usually present (with short base and strong spine); adipose fin usually present and anal fin base very long, not confluent with caudal, 24-90 rays. Pelvic fin is occasionally absent in some species of several genera (Nelson, 1994). This family contains 15 genera with approximately 64 species and almost distribute in fresh-water bodies of Africa and southern Asia (Teugels, 2003). The description for schilbeid genera and species is given below and their distributions in Africa and Asia are shown in Figure 2 and Figure 3, respectively.

1. African schilbeids

In Africa, schilbeids are presently known about 33 species in five genera: *Irvineia* (2 species), *Parailia* (5 species), *Pareutropius* (4 species), *Schilbe*

(21 species) and *Siluranodon* (1 species). They distribute over nearly the entire continent with the exception of the arid regions (Burgess, 1989)

a) *Irvineia*

This genus is characterized by the possession of nine rays in a ventral fin and the prolongation of the swimbladder almost to the very end of the anal fin base (Burgess, 1989). *Irvineia* includes only two species, *I. orientalis* from Juba-Uebi Shebeli system and *I. voltae* from the Volta River in western Africa (Ferraris, 2007).

b) *Parailia*

The genus *Parailia* can be distinguished from the other schilbeid genera by the absence of dorsal fin and teeth on the palate. It contains two subgenera, *Parailia* and *Physailia* which can be distinguished primarily by the presence (*Physailia*) or absence (*Parailia*) of an adipose fin. The subgenus *Parailia* contains only two species, one from the Congo basin, *P. congica* and the other, *P. spiniserratus* from Gambia. The subgenus *Physailia* contains three species from Somaliland (*P. somalensis*), the Congo (*P. occidentalis*) and the Nile basin (*P. pellucid*) (Burgess, 1989).

c) *Pareutropius*

This genus is divided into two subgenera, *Eutropiellus* and *Pareutropius.* The subgenus *Eutropiellus* contains two species, *P. debauwi* and *P. buffei* from the Congo basin and Niger Rivers, respectively. They possess only one pair of mandibular barbels and small size, maximum length 8 cm. The subgenus *Pareutropius* is much like *Eutropiellus* but can be distinguished from that subgenus by having two pairs of mandibular barbels. There are two species in the subgenus *Pareutropius*, *P. longifilis* and *P. mandevillei* from the Congo River (Burgess, 1989 and Ferraris, 2007).

d) *Schilbe*

The genus *Schilbe* is a moderately large genus of African catfishes that contains 21 species which share the characters of dorsal fin with a spine and 5-6 rays and there is a band of villiform teeth on jaws and palate (Teugels, 2003). It is divided into two unequal subgenera, *Schilbe* with three species and *Eutropius* with 18 species (Burgess, 1989) and they can be distinguished primarily by the presence (*Eutropius*) or absence (*Schilbe*) of an adipose fin. The subgenus *Schilbe* contains *S. marmoratus* from the Congo River (Ferraris, 2007), *S. mystus* and *S. uranoscopus* from the Nile River. They can be distinguished from each other by color and by the extent of the anal fin (Burgess, 1989). The subgenus *Eutropius* includes 18 species, *S. angolensis* from the Quanza River in Angola, *S. banguelensis* from Lake Bangweulu, *S. bocagii* from the Bengo River, Angola, *S. brevianalis* from coastal rivers in Nigeria and Cameroon, *S. congensis* from Congo River system, *S. djemeri* from the upper Sanaga River basin, Cameroon, *S. durinii* from Lake Tanganyika, *S. grenfelli* from Congo River basin, *S. intermedius* from Sub-Saharan Africa, *S. laticeps* from Congo River basin, *S. mandibularis* from the St. Paul River, *S. micropogon* from Volta, Gold Coast, *S. moebiusii* from the Rufji and Kingani Rivers, *S. multitaeniatus* from the Dja and Nyong Rivers, *S. nyongensis* from Nyong River, Cameroon, *S. tumbanus* from middle Congo River basin, *S. yangambianus* from Congo River basin and upper Zambezi River and *S. zairensis* from lower Congo River basin (Burgess, 1989; Ferraris, 2007).

e) *Siluranodon*

The genus *Siluranodon* is a small genus containing a single species, S. *auritus* from the Nile and Niger Rivers (Ferraris, 2007). It is characterized by the absence of adipose fin, dorsal fin without spine and no teeth on jaws or palate (Burgess, 1989).

Figure 2 River map of Africa showing the distribution of the African schilbeids.

S1-S33 are assigned for each species: S1 = *Irvineia orientalis*, S2 = *I. voltae*, S3 = *Parailia congica*, S4 = *P. spiniserratus*, S5 = *P. somalensis*, S6 = *P. occidentalis*, S7 = *P. pellucid*, S8 = *Pareutropius debauwi*, S9 = *P. buffei*, S10 = *P. longifilis*, S11 = *P. mandevillei*, S12 = *Schilbe marmoratus*, S13 = *S. mystus*, S14 = *S. uranoscopus*, S15 = *S. angolensis*, S16 = *S. banguelensis*, S17 = *S. bocagii*, S18 = *S. brevianalis*, S19 = *S. congensis*, S20 = *S. djemeri*, S21 = *S. durinii*, S22 = *S. grenfelli*, S23 = *S. intermedius*, S24 = *S. laticeps*, S25 = *S. mandibularis*, S26 = *S. micropogon*, S27 = *S. moebiusii*, S28 = *S. multitaeniatus*, S29 = *S. nyongensis*, S30 = *S. tumbanus*, S31 = *S. yangambianus*, S32 = *S. zairensis*, S33 = *Siluranodon auritus.*

2. Asian schilbeids

In southern and Southeast Asia, schilbeids are presently known about 31 species in 10 genera: *Ailia* (1 species), *Ailiichthys* (1 species), *Clupisoma* (9 species), *Eutropiichthys* (5 species), *Laides* (2 species), *Neotropius* (3 species), *Platytropius* (1 species), *Proeutropiichthys* (4 species), *Pseudeutropius* (3 species) and *Silonia* (2 species). They are mostly distributed in and around India (Burgess, 1989).

a) *Ailia*

The genus *Ailia* is characterized by the absent of dorsal fin and anal fin long, with 59-90 rays. It contains a single species, *A. coila* from northern India (Ferraris, 2007).

b) *Ailiichthys*

This genus also contains only one species, *A. punctata* which there is no ventral fins. It is found in northern India and Pakistan.

c) *Clupisoma*

The genus *Clupisoma* possesses the greatly reduced swimbladder, with thick-walled and flattened. The vomeropalatine teeth may be in a single continuous band or in two or four separate patches. This genus includes nine species (Ferraris, 2004; Chen *et al*., 2005). *C. roosae* from the upper Irrawaddy River in Myanmar differs from other congeners by its shortly abdominal keel. In contrast, the keel of *C. prateri* extends for most of the length of the abdomen. It is known from the lower Irrawaddy and Salween Rivers (Ferraris, 2004). *C. sinense* from the Mekong River in China, Laos and Thailand is similar to *C. roosae* but exhibits a higher number of gill rakers (20-28 vs. 15-17). *C. garua* from the Gangetic region of India and Bangladesh has a fewer branched anal-fin rays (less than 33) than other congeners

(more than 43). *C. montana* from the Teesta River, India and *C. naziri* from Indus River, Pakistan is distinguished from other *Clupisoma* species by the absence of midventral keel along abdomen but *C. montana* has a shorter maxillary barbel than *C. naziri*. *C. bastari* from the Godavari River of Madhya Pradesh has the higher numbers of anal-fin rays (52-54) than other congeners (less than 33-47) (Ferraris, 2004). *C. nujiangense* was recently reported as a new species from the Salween River in China (Chen *et al*., 2005). It is similar to *C. longianalis* from the Mekong River, China but can be differentiated by the nasal barbel which extends slightly past posterior margin of orbit (vs. reaching midpoint of pectoral fin spine) (Chen *et al*., 2005).

d) *Eutropiichthys*

The genus *Eutropiichthys* is distinguished from other schilbeid genera by the presence of an elongate mouth that extends posteriorly at least to the vertical through the anterior margin of the orbit and the palatal teeth arranged in a broadly parabolic patch (Ferraris and Vari, 2007). This genus contains five species. *E. murius* which occurs in the Ganges-Brahmaputra River system can be distinguished from its congeners by the lower number of branched anal-fin rays (32-37 vs. 44-55). *E. vacha* from the rivers of eastern Pakistan, northern India, Nepal, Bhutan and Bangladesh possesses 15-20 gill rakers, a pointed snout and a pectoral spine with roughened anterior margin. *E. burmannicus* which originally proposed as a variety of *E. vacha* was found to be a distinct species with the possession of high numbers of gill rakers (22-28) than *E. vacha* (15-20). It is known from the Irrawaddy and Salween Rivers of Myanmar. There are two additional species, *E. britzi* from the Irrawaddy and Sittang Rivers of Myanmar and *E. salweenensis* from the portion of the Salween River in Thailand. *E. britzi* differs from *E. vacha* in having a rounded snout (vs. pointed snout). *E. salweenensis* can be differentiated from *E. vacha* in having a pectoral spine with smooth anterior margin (vs. roughened in *E. vacha*).

e) *Laides*

The genus *Laides* is characterized by three pairs of barbels (maxillary, mandibular and mental) and vomerine teeth in two separate transverse bands. There are two species, *L. hexanema* and *L. longibarbis* (Ferraris, 2007). *L. hexanema* is known only from Sumatra and Peninsular Malaysia (Ng, 1999). It is diagnosed in having 36-39 anal-fin rays, 34.8-37.9 % SL of anal-fin base 28.6-38.5 % HL of eye diameter and 37.9-47.3 % HL of interorbital distance. The other one species, *L. longibarbis* can be differentiated from *L. hexanema* in having a longer anal-fin base (38.6-41.5 % SL), a smaller eye (eye diameter, 20.1-23.6 % HL) and a larger interorbital distance (47.0-55.6 % HL) and is only known from the Mekong, Mekhlong and Chao Phraya Rivers.

f) *Neotropius*

The genus *Neotropius* is diagnosed by the possession of three separate patches of vomeropalatine teeth and a large, well-developed and thick-walled swimbladder (Burgess, 1989). This genus contains three species including *N. acutirostris* from the Irrawaddy, Sittang and Bago Rivers in Myanmar, *N. atherinoides* from India, Nepal and Bangladesh and *N. khavalchor* from Krishna River, India (Ferraris, 2007).

g) *Platytropius*

The genus *Platytropius* is a small genus containing a single species from Thailand, *P. siamensis.* This species was considered as probably extinct from drainages of Thailand because the last specimen was collected from the Chao Phraya River in 1965 (Vidthayanon and Roongthongbaisuree, 1993).

h) *Proeutropiichthys*

The genus *Proeutropiichthys* is characterized by the presence of vomeropalatine teeth in four distinct patches or in two extensive patches separated in the middle, but not in one continued patch and swimbladder of moderate size. This genus comprises four species, *P. buchanani* from India, *P. goongwaree* from southern India, *P. macropthalmos* from Irrawaddy, Sittang, and Bago River basins, Myanmar and *P. taakree* from India (Burgess, 1989).

i) *Pseudeutropius*

The genus *Pseudeutropius* is diagnosed by the presence of two separate patches of vomeropalatine teeth and a large, thin-walled of swimbladder (Burgess, 1989). This genus contains three species including *P. brachypopterus* from the Kapuas River in Sumatra, *P. mitchelli* from India and *P. moolenburghae* from the Batang Hari River in Sumatra (Ferraris, 2007).

j) *Silonia*

Silonia including two species, *S. childreni* and *S. silondia. S. childreni* originates from the Cauvery, Godavari, and Krishna River basins in India and is characterized in having a terminal mouth, two pairs of barbels with maxillary barbels (long, extend to operculum or slightly beyond) and mandibular barbels (equal to eye-diameter). *S. silondia* distributes in Northern India, Bangladesh, Myanmar, and Nepal. It is similar to *S. childreni* but differs from *S. childreni* in having the mandibular barbels vestigial and embedded in the skin, back with dusky-green color (vs. blue) and abdomen with silver color (vs. white) (Talwar and Jhingran, 1991).

Of all 31 schilbeid species which distribute throughout southern and Southeast Asia, only five species have been found in Thailand (Ferraris, 2007) as follows: *Platytropius siamensis* (Vidthayanon and Roongthongbaisuree, 1993), *Eutropiichthys salweenensis* (Ferraris and Vari, 2007), *Laides longibarbis* (Ng, 1999),

Clupisoma prateri (Vidthayanon and Roongthongbaisuree, 1993) and *C. sinense* (Ferraris, 2007). *Platytropius siamensis* was lastly collected from the Chao Phraya River in 1965 and was considered to be the extinct species (Vidthayanon and Roongthongbaisuree, 1993). *Eutropiichthys salweenensis* was recently recognized as a new species (Ferraris and Vari, 2007). It was previously considered as *E. vacha*. However, based on the taxonomic revision of *E. vacha* (Ferraris and Vari, 2007), the specimens from the Salween River were found to be distinct from the specimens from India, Pakistan and Bangladesh in the combination of the number of rackers on the first gill arch, the number of branched anal-fin rays, the length of the accessory premaxillary tooth patch, the location of the posterior limit of the upper jaw, the extent of the fleshy flap along the anterior margin of the posterior naris, the form of the lateral margin of the pectoral spine and the form of the snout in lateral view. Thus, the Salween specimen which distribute in the Salween River of Thailand was considered to be a valid species and then was named as *E. salweenensis* (Ferraris and Vari, 2007), whereas *E. vacha* is presently known only from Pakistan across India, Nepal and Bangladesh (Ferraris and Vari, 2007). *Laides longibarbis* found in the Mekong and Chao Phraya Rivers of Indochinese was previously considered as a junior synonym of *L. hexanema*. However, based on the taxonomic revision of *L. hexanema* (Ng, 1999), the Indochinese specimens were found to be distinct from the Sundaic specimens with the combination of a longer anal-fin base, a smaller eye and a larger interorbital distance. Thus the Indochinese specimen was recognized to be valid as *L. longibarbis*, whereas *L. hexanema* is presently known only from Sumatra and Peninsular Malaysia. *L. sinensis* was previously placed in the genus *Laides* (Kottelat, 1989; Zakaria-Ismail, 1992), but Roberts (1989) raised possibly that *Laides* could be a synonym of *Clupisoma*. Vidthayanon and Roongthongbaisuree (1993) then considered *L. sinensis* to be *C. sinensis*, chiefly on the basis of the presence of four pairs of barbels (instead of three in *Laides*). Although some authors (e.g. Rainboth, 1996) have retained *L. sinensis* in *Laides*, Ng (1999) indicated that the morphological characteristics of this species are nearer to those of *Clupisoma* species and *L. sinensis* is therefore considered a species of *Clupisoma* as *C. sinensis* or *C. sinense* (Ferraris, 2007).

As mentioned earlier regarding the taxonomy of the family Schilbeidae, two common problems likely contributed to the inconsistency of previous taxonomic studies: limited morphological characteristics and/or overlapped diagnostic features. This is illustrated by the unstable taxonomic status of *C. sinense*. It is superficially similar to the species of *Clupisoma*, especially the presence of nasal barbels (Vidthayanon and Roongthongbaisuree, 1993; Ng, 1999) but its certain morphological characteristics including the characters of the anterior and posterior nostrils, the palatal tooth patches, barbels shape, pelvic fin rays and pectoral fin spine, also overlap with *Laides* species, indicating that it is also potentially more closely related to the species of *Laides* (Kottelat, 1989; Zakaria-Ismail, 1992; Rainboth, 1996). In recent years, based on the extensively morphological and morphometric analysis, several taxonomic revisions of pangasiid and schilbeid taxonomy have been put forward and have led to the recognition of several new species including *H. leptorhynchus* (Pangasiidae), *E. salweenensis* and *L. longibarbis* (Schilbeidae). These putatively new species can be distinguished from their morphologically similar congeners, *H. waandersii*, *E. vacha* and *L. longibarbis*, respectively, by the combination of morphometric features such as length of anal-fin base, length of caudal peduncle, length of head, size of eye (*H. leptorhynchus – H.waandersii*; *L. longibarbis – L. hexanema*), interorbital distance (*L. longibarbis – L. hexanema*) and the length of the accessory premaxillary tooth patch (*E. salweenensis – E. vacha*). These diagnostic characters are somewhat obscure, especially working with the closely related species because they can be mixed (Gustiano *et al*., 2004; Philasamorn and Satrawaha, 2009) and their variability with respect to growth of the specimens (Watanabe *et al*., 2007). Thus to confirm the validity of these putatively new pangasiid and schilbeid species, the independent and different approach is also necessary.

In recent years, the phylogenetic approach based on molecular data, especially DNA sequences has been widely utilized in study of taxonomic resolution (Hillis and Wiens, 2000; Reed *et al*., 2001; Šlechtová *et al*., 2008; Heyden and Matthee, 2008). Because of the instability of pangasiid and schilbeid

classification based on morphological data, the molecular phylogenetic approach will be used in this study in order to clarify the taxonomy of both families.

Figure 3 River map of southern and Southeast Asia showing the distribution of the Asian schilbeids.

S34-S64 are assigned for each species: S34 = *Ailia coila*, S35 = *Ailiichthys punctata*, S36 = *Clupisoma roosae*, S37 = *C. prateri*, S38 = *C. sinense*, S39 = *C. garua*, S40 = *C. montana*, S41 = *C. naziri*, S42 = *C. bastari*, S43 = *C. nujiangense*, S44 = *C. longianalis*, S45 = *Eutropiichthys murius*, S46 = *E. vacha*, S47 = *E. burmannicus*, S48 = *E. britzi*, S49 = *E. salweenensis*, S50 = *Laides hexanema*, S51 = *L. longibarbis*, S52 = *Neotropius acutirostris*, S53 = *N. atherinoides*, S54 = *N. khavalchor*, S55 = *Platytropius siamensis*, S56 = *Proeutropiichthys buchanani*, S57 = *P. goongwaree*, S58 = *P. macropthalmos*, S59 = *P. taakree*, S60 = *Pseudeutropius brachypopterus*, S61 = *P. mitchelli*, S62 = *P. moolenburghae*, S63 = *Silonia children*, S64 = *S. silondia.* S37, S38, S49, S51 and S55 with underline represent schilbeid species in Thailand.

2. Previous phylogenetic relationships of Pangasiidae and Schilbeidae

There is no available for intrafamilial phylogeny within the family Pangasiidae based on morphological data. The first phylogenetic hypothesis was performed by analysis of allozyme and with 18 nominal species of the genera *Helicophagus* and *Pangasius*. By Fitch cluster analysis, the obtained phylogenetic tree indicated that the genus *Pangasius* was polyphyletic but supported *Helicophagus* as monophyletic clade and nested within the *Pangasius* clade. Within the genus *Pangasius*, the putative subgenera *Pangasianodon*, *Neopangasius* and *Pangasius* were also recognized as non-monophyletic (Pouyaud *et al*., 1998).

With the application of the allozyme and a small fragment (539 bp) of the mt *cyt b* sequences, Pouyaud *et al*. (2000) constructed the NJ tree of 20 pangasiid species. The obtained phylogeny confirmed the monophyly of the genus *Helicophagus* and provided support for the recognition of some *Pangasius* subgenera; *Pangasianodon*, *Pteropangasius* and *Pangasius*, except for *Neopangasius* which was recognized as polyphyletic by nesting within the subgenus *Pangasius*. Among the recognized pangasiid groups, the interrelationships were weakly supported. In addition, the intrarelationships within *Neopangasius* + *Pangasius* clade could not be resolved. With respect to pangasiid intrarelationships, a group containing H*elicophagus* and *Pteropangasius* was proposed as the basal group and *Neopangasius* + *Pangasius* as more recent diverged group of pangasiids.

Based on partial mt *12S rRNA* data (737 bp), Pouyaud *et al*. (2004) re-analysed pangasiid phylogeny. The resulting NJ tree revealed that pangasiids were divided into four monophyletic clades which were recognized as four pangasiid genera, *Helicophagus*, *Pangasius*, *Pangasianodon* and *Pteropangasius.* The intergeneric relationships were proposed as (*Pteropangasius*, (*Pangasianodon*, (*Pangasius*, *Helicophagus*))) but with lack of statistical supports for both inter- and intrageneric relationships, especially within *Pangasius*. Inconsistent with the previous study (Pouyaud *et al*., 2000), the *Pteropangasius* was proposed as the most basal group of pangasiids. In the same study with the smaller *12S rRNA* data (527 bp) and

the inclusion of schilbeid taxa for phylogenetic analysis, the incongruent topologies of the Pangasiidae were found. The Pangasiidae and also the genus *Pangasius* became to non-monophyletic assemblage (Pouyaud *et al*., 2004).

The lack of resolution and/or incongruence of recovered pangasiid trees in previous studies might be due to different type of molecular data (allozyme or DNA data) and different selected genes (*cyt b* or *12S rRNA*) which possess different degree of substitution rates (Meyer, 1993). In particular, insufficient phylogenetic informative characters may result in poor resolution of the phylogeny (Crow *et al*., 2004). The previous authors (Pouyaud *et al*., 2000; Pouyaud *et al*., 2004) indicated that using a large variety of molecular characters are urgently needed to resolve and increase the probability of recovering the robust phylogeny. Zardoya and Meyer (1996) suggested that more sampling of sequence data sets would result in an accurate phylogenetic reconstruction of a strong statistical confidence.

Within Schilbeidae, based on morphological data, the cladistic intrarelationships have been proposed either non-monophyletic (Mo, 1991; Teugel, 1996) or monophyletic groups (de Pinna, 1998; Diogo *et al*., 2004). Mo (1991) considered the Schilbeidae to be a non-monophyletic assemblage, with a '*Schilbe* group'representing the real schilbeids, one phylogenetically distinct '*Ailia* group' being closer to the Clariidae and Heteropneustidae and one third, also phylogenetically distinct '*Pseudeutropius* group' being closer to the Bagridae or Pangasiidae. The non-monophyly of Schilbeidae was also supported by Teugels (1996) who proposed that there were no published autapomorphies to support the monophyly of the Schilbeidae, and that, in fact, this family is probably a non-monophyletic assemblage. In contrast, de Pinna (1998) included three different groups of schilbeids in the analysis of the higher-level phylogeny of the Siluriformes, namely *Laides*, Schilbinae and Ailiinae groups. He proposed that the Schilbeidae constitute, in fact, a monophyletic group, which could be diagnosed by a peculiar, unique feature: Meckel's cartilage extending posteriorly much further beyond the limit of dentary-anguloarticular in the coronoid process. In agreement with de Pinna (1998), Diogo *et al*. (2004) supported schilbeid monophyly by defined at least three

autapomorphies features which found only in the representatives of all five schilbeid group (*Schilbe*, *Siluranodon*, *Ailia*, *Pseudeutropius* and *Laides*) but not in other catfish examined.

Very few attempts were made to infer the molecular phylogenetic relationships of the family Schilbeidae. By using the partial sequence of *12S rRNA*, Pouyaud *et al*., 2004 contributed the genetic relatedness of seven species of Asian and African schilbeids. NJ tree demonstrated that the Schilbeidae seems to be a monophyletic group, but with low statistical support (50%). The intrarelationships among those schilbeids were proposed as (*Schilbe*, (*Pseudeutropius*, ((*Clupisoma*, *Laides*), (*Eutropiichthys*, *Silonia*)))). The genus *Laides* formerly placed either in Pangasiidae or in Schilbeidae formed a monophyletic assemblage with other schilbeids and indicated definitely that it belongs to the Schilbeidae. Recently, with MP, ML and BI analyses of nuclear *RAG1* and *RAG2* genes, Sullivan *et al*. (2006) proposed the higher-level relationships among catfishes. The family Schilbeidae was recognized as non-monophyletic with the separation of African schilbeid clade, Asian schilbeid clade (*Laides* + *Ailia*) and another Asian schilbeid genus, *Pseudeutropius* which positioned to the family Horabagridae.

As mentioned earlier regarding to the intrarelationships within schilbeids, the phylogenetic position of *C. sinense* has not been described, thus question also remain about the validity of this species. It is morphologically similar to both the species of *Clupisoma* and *Laides.* Also, the phylogenetic position of the newly described schilbeid species including *L. longibarbis* and *E. salweenensis* has not yet been demonstrated.

In this thesis, phylogenetic relationships of pangasiids and schilbeids will be evaluated with the application of multiple genetic loci including the mt *cyt b*, the contiguous fragment of the posterior half of the *12S rRNA*, the entire $tRNA^{Val}$ and the anterior half of the *16S rRNA* gene and nuclear *RAG1* sequences. Based on more characters of DNA sequences, the resultant phylogenies may help to resolve and increase the probability of recovering the robust phylogeny. In addition, the

phylogenetic position of the newly described species including *H. leptorhynchus* (Pangasiidae), *L. longibarbis* as well as *E. salweenensis* (Schilbeidae) and also the enigmatic *C. sinense* (Schilbeidae) will be assessed and discussed in this study.

3. Estimation of divergence times

A key feature of molecular phylogenies is that not only relationships can be reconstructed, but also that divergence events can be dated using various models of the expected rate of accumulation of mutations in the sequence over time. The idea of dating evolutionary divergences using calibrated sequence distances was first proposed by Zuckerkandl and Pauling (1965) who postulated that the amount of difference between the DNA molecules of two species is a function of the time since their evolutionary separation (Bromham and Penny, 2003). This was termed "molecular clock" and was shown comparing amino acid substitution rates with ages estimated from fossils. The central assumption of the molecular clock is that all branches of a phylogenetic tree evolve at the same, global substitution rate (i.e. there is rate constancy). A clock-like tree is ultrametric (i.e. the total distance between the root and every tip is constant), so nodal depths can be easily dated if the divergence time for at least one node is known (calibration point): the global rate of substitution is calculated and, based on it, divergence times for all nodes can be estimated by linear regression of the molecular distances (Li and Graur, 1991).

There are increasing evidences that the assumption of rate constancy is often violated and that DNA sequence of even closely related species can evolve at different rates (Bromham and Penny, 2003). The reasons given for these deviations from the clock-like model of sequence evolution are related to generation time, metabolic rate, mutation rate and effective population size on the rate of fixation of mutations (Thorne and Kishino, 2002). In practice, clock-like behavior of the data can be tested using the likelihood ratio test (LRT; Felsenstein, 1981) statistic. If the null hypothesis of a constant rate is rejected, the use of methods that try to change model rate over the tree (so-called "relaxed clock methods") is necessary. There are many such methods that use different approaches to either correct or incorporate rate

heterogeneity in the dating process on the basis of specific rate change models. Of all these methods, the Bayesian rate autocorrelation dating (Kishino *et al*., 2001) is becoming increasingly popular. This method uses a fully probabilistic and high parametric model to describe the change in evolutionary rate over time and uses MCMC approximation to derive the posterior distribution of rates and times from a prior distribution. For the assignments of rates to different branches in the tree, rates are drawn from a lognormal distribution and a parameter called Brownian motion constant describes the amount of autocorrelation (Kishino *et al*., 2001). In order to scale rates and times, the prospective age of the root node must be specified *a priori*. This method provides Bayesian credibility intervals for estimated divergence times and substitution rates and allows multiple calibration constraints on nodes (specified as prior age intervals) which usually determined from fossils. Bayesian dating method is able to account for multiple genes/loci with different evolutionary behaviours. This simultaneous analysis of multiple genes may yield more accurate estimates of divergence times (Thorne and Kishino, 2002).

MATERIALS AND METHODS

1. Taxon sampling and species classification

A total of 11 extant pangasiid species (22 individuals) and four extant schilbeid species (eight individuals) inhabiting in Thailand were obtained from the local fish markets. Another two species of pangasiids (*Pangasius nasutus* and *Helicophagus typus*), and two of schilbeids (*Pseudeutropius brachypopterus* and *P. moolenburghae*) which are native to Indonesia were supplied by Dr. Tan Heok Hui, Department of Biological Sciences, National University of Singapore. Sampling localities of the specimens are shown in Table 2.

Species of pangasiid and schilbeid catfishes in this study were classified according to Roberts and Vidthayanon (1991) and Vidthayanon and Roongthongbaisuree (1993), based on the characteristics of head shape, barbels, nostrils, mouth and dentition, swimbladders, gill rakers, fins and color patterns. The generic and species names of pangasiids and schilbeids were primarily based on Vidthayanon and Roongthongbaisuree (1993).

2. DNA extraction, molecular marker, PCR amplification, PCR product purification and nucleotide sequencing

A. DNA extraction

All specimens were cut into small pieces with the sterile scalpel blades and the muscle or fin clips were put in 1.5 ml microcentrifuge tubes, and subsequently stored at either -80 °C or in 70% ethanol. The whole fish was likewise preserved at either -80 °C or in 70-100% ethanol for further use. Whole genomic DNA was extracted from approximately 0.05-0.1 g of fresh or ethanol-preserved white muscle or fin tissue using the AquaPure Genomic DNA Isolation Kit (Bio-Rad) according to the manufacturer's protocol. The extracted DNA in total volume of 100 µl of the TE buffer was stored at -20ºC in a refrigerator.

B. Molecular marker

1. Mitochondrial (mt) DNA

MtDNA genes have been widely used in animal molecular phylogenetic study for several reasons; their relatively small size (approximately 16 kb) and their high copy number. The mutation rate of animal mtDNA is about tenfold value of nuclear DNA. These characteristics are useful for assessing genetic relationships of individuals or groups within a species and also for assessing the phylogeny among different species. In addition, maternal inheritance and absence of recombination make mtDNA a powerful tool for tracking back family tree down the maternal line (Brown *et al*., 1982; Thorne *et al*., 1998; Avise, 1994). For these reasons, mtDNA has been used for phylogenetic studies in various groups of animal such as birds (Härlid *et al*., 1997), mammals (Castresana, 2001; Cabria *et al*., 2006), caecilian amphibians (San Mauro *et al*., 2004), lizards (Raselimanana *et al*., 2009) as well as fishes (Zardoya and Meyer, 1996; Song *et al.*, 1998; Orrell and Carpenter, 2004; Heyden and Matthee, 2008; Doadrio *et al*., 2009). However, different regions of mtDNA genome; protein coding region, rRNA gene and tRNA, possess different degree of substitution rates (Table 3) because these genes have different functional and structural constraints, allowing suitable regions to be chosen for the question under studies (among higher level, between recently divergent groups, populations, species and even individuals) (Brown *et al*., 1982; Thorne *et al*., 1998). Therefore, the choice of specific genes that are most appropriate for the phylogenetic question at hand is a crucial step, as the results of the study are largely dependent on the selected gene (San Mauro, 2006). Over the years, mt *cyt b* and ribosomal genes have long been used in animal phylogenetic studies at various taxonomic levels (Orrell and Carpenter, 2004).

Table 2 Summary of specimens, identification number (ID) for individuals of each species, GenBank Accession numbers and sample locations of pangasiids and schilbeids used in this study.

Family/Species	ID	GenBank Accession Number			Sampling localities
		Cyt b	RNA	*RAG1*	
Pangasiidae					
Pangasius bocourti	01	GQ856793	HM355769	HM355781	Mekong river: Ubonratchathani, Thailand
Pangasius bocourti	02	GQ856794	HM355770	HM355782	Mekong river: Ubonratchathani, Thailand
Pangasius conchophilus	01	HM236385	HM355771	HM355786	Chao Phraya river: Ayudhya, Thailand
Pangasius conchophilus	02	HM236385	HM355772	HM355786	Chao Phraya river: Ayudhya, Thailand
Pangasius krempfi	01	HM236386	HM355773	HM355787	Mekong river: Ubonratchathani, Thailand
Pangasius krempfi	02	HM236390	HM355773	HM355787	Mekong river: Ubonratchathani, Thailand
Pangasius larnaudii	01	HM236391	HM355774	HM355788	Chao Phraya river: Nakhonsawan, Thailand
Pangasius larnaudii	02	HM236392	HM355775	HM355788	Chao Phraya river: Ayudhya, Thailand
Pangasius macronema	01	HM236393	HM355776	HM355783	Mekong river: Ubonratchathani, Thailand
Pangasius macronema	02	HM236394	HM355777	HM355784	Chao Phraya river: Ayudhya, Thailand
Pangasius nasutus	01	HM236395	HM355778	HM355789	Kalimantan Barat: Kapuas, Indonesia
Pangasius nasutus	02	HM236396	HM355778	HM355789	Kalimantan Barat: Kapuas, Indonesia
Pangasius polyuranodon	01	HM236399	HM355779	HM355785	Chao Phraya river: Ayudhya, Thailand
Pangasius polyuranodon	02	HM236399	HM355779	HM355785	Chao Phraya river: Ayudhya, Thailand
Pangasius sanitwongsei	01	HM236400	HM355780	HM355790	Chao Phraya river: Nakhonsawan, Thailand

Table 2 (Continued)

Family/Species	ID	GenBank Accession Number			Sampling localities
		Cyt b	RNA	*RAG1*	
Pangasiidae					
Pangasius sanitwongsei	02	HM236401	HM355780	HM355790	Chao Phraya river: Nakhonsawan, Thailand
Pseudolais pleurotaenia	01	HM236397	HM355765	HM355791	Chao Phraya river: Ayudhya, Thailand
Pseudolais pleurotaenia	02	HM236398	HM355766	HM355791	Mekong river: Ubonratchathani, Thailand
Pangasianodon hypophthalmus	01	GQ856796	HM355767	HM355792	Mekong river: Ubonratchathani, Thailand
Pangasianodon hypophthalmus	02	GQ856796	HM355767	HM355792	Mekong river: Ubonratchathani, Thailand
Pangasianodon gigas	01	GQ856795	HM355768	HM355793	Mekong river: Ubonratchathani, Thailand
Pangasianodon gigas	02	GQ856795	HM355768	HM355793	Mekong river: Ubonratchathani, Thailand
Helicophagus leptorhynchus	01	GQ856791	HM355763	HM355794	Mekong river: Ubonratchathani, Thailand
Helicophagus leptorhynchus	02	GQ856792	HM355763	HM355794	Mekong river: Ubonratchathani, Thailand
Helicophagus typus	-	GQ856790	HM355764	HM355795	Sumatra: Jambi, Indonesia
Schilbeidae					
Clupisoma sinense	01	HM236379	HM355754	HM355801	Mekong river: Ubonratchathani, Thailand
Clupisoma sinense	02	HM236380	HM355755	HM355802	Mekong river: Ubonratchathani, Thailand
Clupisoma prateri	01	HM236378	HM355752	HM355798	Salween river: Maehongson, Thailand
Clupisoma prateri	02	HM236378	HM355753	HM355798	Salween river: Maehongson, Thailand
Laides longibarbis	01	HM355762	HM355758	HM355796	Mekong river: Ubonratchathani, Thailand

Table 2 (Continued)

Family/Species	ID	GenBank Accession Number			Sampling localities
		Cyt b	RNA	*RAG1*	
Schilbeidae					
Laides longibarbis	02	HM355762	HM355759	HM355797	Mekong river: Ubonratchathani, Thailand
Eutropiichthys salweenensis	01	HM236381	HM355756	HM355799	Salween river: Maehongson, Thailand
Eutropiichthys salweenensis	02	HM236382	HM355757	HM355800	Salween river: Maehongson, Thailand
Pseudeutropius brachypopterus	-	HM236383	HM355760	HM355804	Sumatra: Jambi, Indonesia
Pseudeutropius moolenburghae	-	HM236384	HM355761	HM355803	Kalimantan Tengah: Kahayan basin, Indonesia

In this study, two mt loci; *cyt b* gene and the contiguous fragment of *12S rRNA*, $tRNA^{Val}$ and *16S rRNA* gene (hereafter referred to as RNA data set), were selected to reconstruct phylogenetic relationships of pangasiids and schilbeids. The *cyt b* has proven to be a robust evolutionary marker for the determination of the phylogenies at various taxonomic levels in fishes. At the higher taxonomic level, *cyt b* was informative in actinopterygian phylogenetic relationships (Lydeard and Roe, 1997). Also, by using *cyt b* sequences, the well-resolved phylogenetic relationships among non-diplomystid catfishes (Siluriformes) were contributed (Hardman, 2005). The well-resolved *cyt b* phylogeny of *Noturus* catfishes supported the recognition of three subgenera, *Rabida*, *Schilbeodes* and *Noturus* which was concordant with previous morphological evidences (Hardman, 2004). *Cyt b* is also useful in resolving phylogenetic relationships of the closely related taxa. *Cyt b* phylogeny of the Chinese spiny loach (*Cobitis sinensis*) highly supported two major clades, which might originate from two different continental populations since the island's initial isolation in the Pliocene (Chiang *et al*., 2010). Several previous studies revealed that *cyt b* is also appropriate to resolve evolutionary relationships at the familial level of fishes such as the families Percidae (Song *et al.*, 1998), Hexagrammidae (Crow *et al*., 2004), Goodeidae (Doadrio and Domínguez, 2004), Sparidae (Orrell and Carpenter, 2004), Sisoridae (Peng *et al*., 2004), Badidae (Rüber *et al*., 2004), Percidae (Sloss *et al*., 2004), Bagridae (Ku *et al*., 2007) as well as Cobitidae (Perdices *et al*., 2008; Šlechtová *et al*., 2008). Zardoya and Meyer (1996) indicated *cyt b* as a good "phylogenetic performer" among phylogenetically distant relatives. For reasons mentioned above, *cyt b* gene was selected as a molecular marker for this study.

The mt ribosomal genes evolve at a faster rate than nuclear ribosomal genes do (Hillis and Dixon, 1991, Meyer 1993), but slower than mt protein coding genes (Table 3). Due to the slower evolutionary rate and reasonably conserved among vertebrates, they are typically used for higher level analyses such as family, superfamily, suborder and order levels. Numerous studies have also used 12S/16S mtDNA sequences to resolve evolutionary relationships of fishes at the familial level such as the families Hexagrammidae (Crow *et al*., 2004), Doradidae (Moyer *et al*.,

2004), Sparidae (Orrell and Carpenter, 2004), Callichthyidae (Shimabukuro-Dias *et al*., 2004) and Percidae (Sloss *et al*., 2004)**.**

Table 3 Substitution rates of certain mitochondrial genes

Gene	Taxa	Substitution rate (% /site/million years)	References
Cyt b	Freshwater fishes	0.84-1.52	Bermingham *et al*. (1997); Zardoya and Doadrio (1999); Machordom and Doadrio (2001); Perdices and Doadrio (2001); Peng *et al*. (2002)
ATPase 6/8	Marine fishes Freshwater fishes	1.3 0.84	Bermingham *et al*. (1997) Perdices *et al*., 2003
12S rRNA	Mammals	0.34	Pesole *et al*., 1999
16S rRNA	Electric fishes Mammals	0.23 0.49	Alves-Gomes (1999) Pesole *et al*., 1999
COI	Marine fishes	1.2	Bermingham *et al*. (1997)
ND2	Marine fishes	1.3	Bermingham *et al*. (1997)
ND1/ND2	Amphibian and reptiles	1.3	Macey *et al*. (1998)

2. Nuclear DNA

Although the mt genes are particularly popular choice for phylogenetic study, several recent studies have utilized some orthologous nuclear protein coding genes for the inference of phylogenetic relationships to provide independent data from different genomes (Groth and Barrowclough, 1999; Quenouille *et al*., 2004; Rüber *et al*., 2004; Šlechtová *et al*., 2008). Various nuclear protein coding genes such as *EF1*α (Moyer *et al*., 2004), *Tmo-4C4* (Rüber *et al*., 2004), *RAG1* (Rüber *et al*., 2004; Sullivan *et al*., 2006; Perdices *et al*., 2008), *myh6* (Li *et al*., 2010), *RH* as well as *GH*

genes (Chen and Mayden, 2009) have been utilized in molecular phylogenetics studies of fishes. Of these, one of the most widely used is the *RAG1* (Sullivan *et al*., 2006).

The *RAG1* (*Recombination Activating Gene 1*) gene is a single-copy gene. It is present in all jawed vertebrates and codes for components of the recombinase which involves in the *V* (*D*) *J* recombination of T-receptor and immunoglobulin genes (Schatz, 2004). This gene has many properties desirable for molecular phylogenetic analyses (Groth and Barrowclough, 1999; Martin, 1999). Indels are rare and the few that exist did not cause alignment problem. A lack of site saturation, even at third positions of codon is found in this gene; consequently, eliminating partitions is not necessary. The base composition in *RAG1* is highly stationary, thus it would not negatively influence phylogenetic inference (Groth and Barrowclough, 1999). Substitution rate of *RAG1* gene was relatively slower than those of almost all mt protein coding genes (San Mauro *et al*., 2004), being similar to those of the sequences of the most conservative mt protein coding genes (cytochrome oxidase subunits, Zardoya and Meyer, 1996), thus it is typically used for higher level analyses such as family, superfamily, suborder and order levels (San Mauro *et al*., 2004). In fishes, *RAG1* appears to be useful in elucidating phylogeny at the familial level such as the families Badidae (Rúber *et al*., 2004), Pomacentridae (Quenouille *et al*., 2004), Synbranchidae (Perdices *et al*., 2005) as well as Cobitidae (Perdices *et al*., 2008; Šlechtová *et al*., 2008).

C. PCR amplification

The mt *cyt b* gene was PCR amplified using a combination of primer L15058 (Kocher *et al*., 1989) and either primer H16249 (Kocher *et al*., 1989) or primer DonThrR (San Mauro *et al*., 2004).

The mt RNA fragment was obtained by PCR amplification of three overlapping fragments using primers L1091 and H1478 (Kocher *et al*., 1989), Amp-

12SF (San Mauro *et al*., 2004) and gob-16SR1 (Rüber *et al*. 2003), and 16Sar-L and 16Sbr-H (Palumbi *et al*., 1991), respectively (Table 4).

A fragment of the nuclear *RAG1* gene was amplified using primers Amp-RAG1F and Amp-RAG1R1 (San Mauro *et al*. 2004). The sequences of primers used in this study are listed in Table 4.

Table 4 PCR primers used for amplification and sequencing.

Fragment name	Primer name	Sequence	Source
Cyt b	L15058	5'-TGACTT GAAA(AC)CCACCGT TG-3'	Kocher *et al*. (1989)
	H16249	5'-TCAGTCTCCGGT TTACAAGACC-3'	
	DonThrR	5'-ACCTCCGATCTTCGGATTACAAGA CCG-3'	San Mauro *et al*. (2004)
12S	L1091	5'-AAAAAGCTT CAAACT GGGATT AGATACCCCACTAT-3'	Kocher *et al*. (1989)
	H1478	5'-TGACTGCAGAGGGTGACGGGCG GTGTGT-3'	
16S	16Sar-L	5'-CGCCTGTTTATCAAAAAC AT-3'	Palumbi *et al*. (1991)
	16Sbr-H	5'-CCGGTCTGAACT CAGATCACG T-3'	
Mid *12S*-*16S*	Amp-12SF	5'-AAGAAATGGGCTACATTT TCT-3'	San Mauro *et al*. (2004)
	gob-16SR1	5'-AAGTGATTGCGCTACCTT CGCAC-3'	Rüber *et al*. (2003)
	gob-12SF2	5'-GTCTCTGTGGCAAAAGAG T-3'	Rüber *et al*. (2003)
RAG1	Amp-RAG1F	5'-AGCTGCAG(CT)CA(AG)TACCA(CT)A A(AG)ATGTA-3'	San Mauro *et al*. (2004)
	Amp-RAG1R	5'-AACTCAGCTGCATT(GT)CCAAT(AG) TCACA-3'	

PCR amplifications were performed in 25 µl reaction mixture comprised 18.4 µl distilled H_2O, 2.5 µl 10x standard reaction buffer (Biotools, Spain), 1 µl dNTPs (2.5 mM), 0.4 µl of each primer (10 µM), 0.3 µl of *Taq* DNA polymerases (Biotools, Spain), and 1 µl of DNA stock (10-100 ng), with the cycling conditions shown in Table 5.

Table 5 PCR cycling conditions for amplifying each PCR fragment.

PCR cycling conditions	PCR fragments									
	Cyt b		RNA						*RAG1*	
			12S		mid *12S-16S*		*16S*			
	Temp (ºC)	Time	Temp (ºC)	Time	Temp (ºC)	Time	Temp (ºC)	Time	Temp (ºC)	Time
Initial denaturation	94	5 min	94	5 min	94	5 min	94	5 min	94	5 min
Denaturation	94	1 min	94	1 min	94	1 min	94	1 min	94	1 min
Annealing	48-50	1 min	54	1 min	54-56	1 min	51	1 min	56	1 min
Elongation	72	1 min	72	45s	72	90s	72	1 min	72	1 min
Final elongation	72	7 min	72	7 min	72	7 min	72	7 min	72	7 min

All of PCR amplifications were done with 35 cycles of denaturation, annealing and elongation steps.

Amplification products were checked by electrophoresis on 1% agarose gel, comparing with GeneRuler 1 kb DNA Ladder (Fermentas). DNA was run in the electrophoresis tank containing 1X TAE buffer, with the voltage of 100 volts for 30 min. The gel was stained with 0.5 µg/ml ethidium bromide for 15 min and then examined under ultraviolet light of UV gel documentation to visualize DNA bands. The resultant DNA band sizes were estimated by DNA ladder (Gene Ruler 1 kb DNA Ladder, Fermentas).

D. PCR product purification and nucleotide sequencing

The PCR products were purified either with an ethanol precipitation or using the purification Kit (QIAquick PCR purification Kit and Gel/PCR DNA Fragments Extraction Kit, Geneaid) according to the manufacturer's protocol. Ethanol precipitation was done by transferring the PCR product to 1.5 µl microcentrifuge tube containing ddH_2O 80 µl, then added 3 M sodium acetate 10 µl and 2 volumes of absolute ethanol. The tube was gently inverted 2-3 times before spining in microcentrifuge (13,000 rpm) for 10 mins at 4 °C. The supernatant was decanted and the DNA was washed with 2 volumes of 70% ethanol and spun for 10 mins. Finally, the DNA pellet was air dried before resuspended in 22 µl of water or TE buffer.

The purified of PCR product was sequenced by an automated DNA sequencer (ABI PRISM 3700) at Secugen S.L. (Madrid, Spain) with PCR primers and internal primer (gob-12SF2 for RNA segment) (Table 5).

3. Sequence assembling and phylogenetic analysis

A. DNA sequence assembly and multiple alignment

The identity of the newly amplified sequences was determined through BLAST searches against GenBank (http://blast.ncbi.nlm.nih.gov/blast.cgi). Each base of all sequences was checked and was visually edited on Chromas program v. 2.33 and then edited sequences from each primer (forward and reverse primers) were assembled using the CAP3 program (http://pbil.univ-lyon1.fr/cap3.php; Huang and Madan, 1999). Complete nucleotide sequences of each of the three data sets (*cyt b*, RNA, and *RAG1*) were aligned independently using MEGA v. 4.0 (Kumar *et al*., 2008). Each resulting alignment was further visually refined, and gapped positions were eliminated. The catfish, *Ictalurus punctatus* (Accession number: AF482987; Waldbieser, 2003) of the related family Ictaluridae was used as outgroup. Output files

in nexus (*.nxs) and phylip (*.phy) format were constructed for further analysis by other programs.

B. Measuring of base composition and Chi-square test of homogeneity of base frequencies

The overall base composition of each nucleotide data set (*cyt b*, RNA and *RAG1*) was estimated using PAUP* v. 4.0b10 (Swofford, 2002). A chi-square ($\chi 2$) test of base composition homogeneity was calculated for all codon positions, as implemented in PAUP* v. 4.0b10. In *cyt b* and *RAG1* data set, measuring of base composition and testing base composition homogeneity were also performed for individual codon (first, second and third) positions.

C. Evolutionary model selection

The program Modeltest v. 3.7 (Posada and Crandall, 1998), based on the Akaike Information Criterion (AIC) was used to select the best evolutionary model of nucleotide substitution that best fit for each nucleotide data set. The evolutionary model was specified for reconstructing phylogeny in NJ, ML and BI analyses and estimating the genetic distances. Specifing evolutionary models of nucleotide substitution would avoid an underestimate of the number of nucleotide substitution because it does not take into account multiple, backward and parallel substitutions.

Each nucleotide data set in nexus format and the command file (modelblock) which deposits in Modeltest folder were executed in PAUP* v. 4.0b10 to obtain the likelihood score which is the input of Modeltest. PAUP* would be started to test the data against 56 different models. Once was finished a score file (model.scores) would be appeared in the same directory as the command file. The best model was received after running the file model.scores through Modeltest.

D. Substitution saturation test

Nucleotide saturation analysis was used to test the occurrence of multiple hits in which two or more mutations take place at the same site in the sequences. Thus, the similarities between two randomly chosen aligned DNA sequences could be the result of chance alone rather than homology (i.e. common ancestry). When the multiple hits occur or the saturation is reached, the phylogenetic signal is consequentially lost (Salemi and Vandamme, 2003).

In the present study, nucleotide saturation was analyzed for each nucleotide data set by means of plotting the total number of transitions (Ts), transversion (Tv) and Ts+Tv (Y axis) against pairwise genetic distances (X axis) which were based on alternative evolutionary models suggested by Modeltest v. 3.7 (Posada and Crandall, 1998). Numbers of nucleotide substitutions (Ts, Tv and Ts+Tv) for each pair of all specimens were counted by using MEGA v.4.0 (Kumar *et al*., 2008). Pairwise genetic distance between all taxa was calculated using PAUP* v. 4.0b10 (Swofford, 2002). A graph was plotted by the values of number of nucleotide difference (Y axis) and genetic distance (X axis) in Microsoft Office Excel 2007.

E. Sequence divergence analysis

The simplest approach to measure the divergence between two strands of aligned DNA sequences is to count the number of sites where they differ. The proportion of different homologous sites is called observed distance or *p*-distance which is expressed as the number of nucleotide differences per site (Strimmer and von Haeseler, 2003). Although the *p*-distance is an underestimation of the true genetic distance because some of the aligned nucleotides are the results of multiple events (Strimmer and von Haeseler, 2003), several previous studies used the sequence divergence (*p*-distance) values as the rough criterion to indicate taxonomic ranks at various levels (family, genus and species) of fishes (Johns and Avise, 1998; Doadrio and Domínguez, 2004; Heyden and Matthee, 2008; Rocha *et al*., 2008; Doadrio *et al*., 2009).

In this study, the sequence divergences (*p*-distances) for each genetic marker were determined by using MEGA v. 4.0 (Kumar *et al*., 2008). The values were compared with previously published papers (e.g. Ritchie *et al*., 1996; Johns and Avise, 1998; Doadrio and Domínguez, 2004; Miller and Cribb, 2007; Almada *et al*., 2008; Heyden and Matthee, 2008; Rocha *et al*., 2008; Doadrio *et al*., 2009) and were used as the criterion to delimit taxonomic ranks (familial, generic and specific levels) of pangasiid and schilbeid catfishes. Also, the species status of putatively new species including *Helicophagus leptorhynchus*, *Laides longibarbis* and *Eutropiichthys salweenensis* which were previously recognized as *H. waandersii*, *L. hexanema* and *E. vacha*, respectively, was validated by means of sequence divergences. Since *H. waandersii*, *L. hexanema* and *E. vacha* distribute beyond the borders of Thailand, thus their DNA sequences were retrived from the GenBank database as follows: *cyt b* for *H. waandersii* (DQ119468; Hardman, 2005) as well as *L. hexanema* (EU490915) and *16S rRNA* for *E. vacha* (GQ357917) for determining sequence divergences.

F. Incongruence Length Difference (ILD) test for combining of nucleotide sequences

In recent years, several studies (Cummings *et al*., 1995; Zardoya and Meyer, 1996; Rokas *et al*., 2003; Crespi and Fulton, 2004; Crow *et al*., 2004; Shimabukuro-Dias *et al*., 2004; Li *et al*., 2010) have demonstrated the need to establish the phylogenetic inferences based on rather large sequence data sets in order to achieve statistical confidence and to obtain the robust phylogeny. In the present study, three genetic loci including the mt *cyt b* gene, the RNA fragment as well as the nuclear *RAG1* gene were combined and used to reconstruct phylogenetic relationships of pangasiids and schilbeids.

Prior to the combination of all data sets, the ILD test (Farris *et al*., 1995) was used to examine possible incongruence between different combinations of genes (*cyt b* vs. RNA, *cyt b* vs. *RAG1*, RNA vs. *RAG1* and mtDNA vs. nuDNA). The ILD test was performed by means of the partition homogeneity test (HOMPART command) in PAUP* v. 4.0b10 (Swofford, 2002) with 1000 replications.

G. Phylogenetic analyses

Cyt b, RNA, *RAG1* and combined data sets were used to infer phylogenetic relationships of all 35 specimens of Pangasiidae and Schilbeidae and one outgroup species, *Ictalurus punctatus* (AF482987; Waldbieser, 2003). For the *cyt b* data set, two additionally sequences of pangasiid species, *H. waandersii* (DQ119468; Hardman, 2005) and schilbeid species, *L. hexanema* (EU490915) were included into the data set for phylogenetic analyses to determine the phylogenetic position of the newly described species in Thailand, *H. leptorhynchus* (Pangasiidae) and *L. longibarbis* (Schilbeidae). Also, the phylogenetic position of the other newly described schilbeid species, *E. salweenensis* was determined and thus the available *16S rRNA* gene of its congener, *E. vacha* (GQ357917) was included for inferring phylogenetic relationships. Because the *16S rRNA* sequence of *E. vacha* is relatively short (465 bp) compared with the RNA data set in this study (approximately 1,850 bp), thus all of the RNA sequences from this study were trimmed to the size of the smallest fragment (465 bp) to maintain consistency of the data and this *16S rRNA* data set was additionally analysed.

A total of five data sets (*cyt b*, RNA, *16S rRNA*, *RAG1* and combined data sets) were analyzed by four well known methods including neighbor joining (NJ; Saitou and Nei, 1987), maximum parsimony (MP; Fitch, 1971), maximum likelihood (ML; Felsenstein, 1981), and Bayesian inference (BI; Huelsenbeck *et al*., 2001).

NJ is one of the distance-based methods. This method converts aligned sequences into a pairwise distance matrix and then puts the matrix into a tree building method (Page and Holmes, 1998). In this study, NJ analyses were implemented in PAUP* v. 4.0b10 (Swofford, 2002) starting from NJ tree searches. Tree topologies were postulated in accordance with the best-fit evolutionary models which were selected by Modeltest v. 3.7 for each data set. Non-parametric bootstrap analyses with 1000 pseudoreplicates were performed to obtain estimates of support for each node of the NJ tree.

MP is one of the character-based or discrete methods which operate directly on DNA sequences or character data rather than on pairwise distance. Thus loss of information when characters are converted to distances is avoided here (Page and Holmes, 1998). In this study, MP analyses were conducted with PAUP* v. 4.0b10 (Swofford, 2002) using heuristic search with 10 random addition sequences and TBR branch swapping. The reliabilities of the MP trees were tested with non-parametric bootstrapping with 1000 pseudoreplicates.

The other one character-based method is ML (Nei and Kumar, 2000). In this study, ML analyses were performed with PHYML v. 2.4.4 (Guindon and Gascuel, 2003) based on the best-fit substitution models which were selected by Modeltest v. 3.7. The reliabilities of the ML trees were tested with non-parametric bootstrapping with 500 pseudoreplicates.

BI is also the character-based method (Nei and Kumar, 2000). In this study, BI analyses were performed using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003). Four simultaneous Metropolis coupled MCMC chains were ran for one million generations with trees sampling every 100 generations (10000 total trees). All sample points prior to reaching convergence (1000 trees) were discarded as burn-in. For the combined data set, model parameters were treated independently from the three data partitions (*cyt b*, RNA, and *RAG1* genes) by the application of the 'unlink' command. Nodal statistical confidence of BI topologies was determined based on the values of Bayesian posterior probability (BPP) obtained from a majority-rule consensus tree.

Since the actual evolutionary relationship of the organisms is almost always unknown, thus it is difficult to decide that which methods are better than others. It is important to realize that every methods has some strengths and some weaknesses (Table 6) and none of the methods is almighty (Nei and Kumar, 2000).

Table 6 Advantages and disadvantages of each phylogenetic analysed method

Method	Advantage	Disadvantage
NJ	- Relatively fast (compared to all other methods available) - Performs well when the divergence between sequences is low	- Loss of information when the sequences are convert to distances - The difficulty in obtaining reliable estimates of pairwise distances for highly divergent sequences
MP	- It is fast enough for the analysis of large data sets containing many sequences - It is robust if branches of the tree are short (either because sequences are closely related or the taxon sampling is dense)	- It can perform poorly (even seriously misleading) if there is substantial variation in rates of evolution among taxa - Cannot incorporate explicit models of sequence evolution , thus it is difficult to deal with high degree of homoplasy when markedly divergent sequences are analysed - only informative sites are used
ML	- Can incorporate explicit models of sequence evolution (including the ability to estimate model parameters, hence allowing simultaneous inference of patterns and processes of molecular evolution)	- It is computationally very long and slow - The result is especially dependent on the correctness of the employed model of sequence evolution
BI	- It is closely allied with ML but being faster and computationally less requiring using equally (or even more) complex models of sequence	- Prior distributions for parameters must be specified, and that it can be difficult to determine whether the MCMC approximation has run for a

Table 6 (Continued)

Method	Advantage	Disadvantage
BI	- Can implement models of sequence evolution for different partitions of sequence data set	sufficient number of cycles

4. Estimation of divergence times

The times of divergence of pangasiids and schilbeids were estimated using Bayesian approaches implemented in BEAST v.1.6.1 (Drummond and Rambaut, 2007) by analyzing the concatenated data set. The BEAST input file was properly created with BEAUti utility included in the same program package. Substitution model parameters were specified for each data partition. A relaxed clock with an uncorrelated lognormal distribution and a speciation Yule process as tree prior were specified. The reference fossil, the appearance of the pangasiid genus *Cetopangasius* in the Miocene (5-10 MY; Roberts and Jumnongthai, 1999) was used to estimate divergence times. The calibration was assigned at the basal node of pangasiids and was treated as a normal prior distribution with a normal mean at 7.5 MY and a normal stdev of 1.27. The analysis was run for 10^7 generations sampled every 1000 steps. The posterior sample was examined in Tracer v.1.5. A burn-in of 25% of all sampled trees was discarded. The final tree with divergence estimates and their 95% highest posterior densities (HPD), representing the range of time, was computed in TreeAnnotator v.1.6.1. FigTree v.1.3.1 enabled the visualization of the attained chronogram.

RESULTS AND DISCUSSION

1. Classification of Thai pangasiids and schilbeids

Pangasiid and schilbeid specimens; obtained from the Chao Phraya, the Mekong and the Salween Rivers, were classified according to characteristics described by Roberts and Vidthayanon (1991) and Vidthayanon and Roongthongbaisuree (1993). In general, they are based on the characteristics of head shape, barbels, nostrils, mouth and dentition, swimbladders, abdomen, gill rakers and fin rays.

Differentiation of Thai pangasiids and schilbeids employed the number of barbels, the number of swimbladder chambers and the operculum shape. Pangasiid species possess two pairs of barbels, maxillary and mandibular; one to four chambers of an elongated swimbladder and the lower posterior border of the operculum is not pointed, while schilbeid species have three to four pairs of barbels, one nasal (absent only in *Laides*), one maxillary and two mandibular pairs; the greatly reduced swimbladder, with a single chamber and pointed lower posterior border of the operculum.

A. The family Pangasiidae

According to the characteristics of head shape, mouth and dentition, abdomen, fin rays, gill rakers and swimbladder, Thai pangasiids were classified into four generanamely, *Helicophagus*, *Pseudolais*, *Pangasianodon* and *Pangasius*. The genus *Helicophagus* comprise a single species of *H. leptorhynchus* (Figure 4A) which is diagnosed in having the much narrow mouth and snout and the absence of palatine tooth patches. The genus *Pseudolais*, also contains only one species, i.e., *P. pleurotaenia* (Figure 4B), characterized by its possession of an entirely keeled abdomen and a four-chambered swimbladder. The *Pangasianodon* is defined by having a single-chambered swimbladder and 8-9 pelvic fin rays instead of the usual six in other pangasiids. The genus contains two species, *P. hypophthalmus* (Figure

4C) with a swimbladder extended beyond the abdomen to the base of an anal fin and *P. gigas* (Figure 4D) with a swimbladder confined to an abdominal cavity. Finally, the genus *Pangasius* with six pelvic fin rays, two or three-chambered swimbladders and an abdomen rounded anterior to the pelvic fins. This genus includes the remaining seven species (Figure 4E – 4K); *P. bocourti*, *P. conchophilus*, *P. krempfi*, *P. larnaudii*, *P. macronema*, *P. polyuranodon* and *P. sanitwongsei*. Each *Pangasius* species is characterized by the combination of swimbladder, palatal teeth and gill rakers. *P. bocourti*, *P. conchophilus*, *P. larnaudii* and *P. sanitwongsei* have two-chambered swimbladders. *P. bocourti* has the numerous gill rakers (35-48). *P. conchophilus* possesses 15-19 gill rakers. *P. larnaudii* is defined by having a large, black humeral spot and *P. sanitwongsei* is diagnosed in having filamentously extension of dorsal, pectoral, pelvic and anal fins. *P. krempfi*, *P. macronema* and *P. polyuranodon* have three-chambered swimbladders. *P. krempfi* has a relatively elongated snout, two crescentic patches of palatal teeth and gill rakers less than 25. *P. macronema* is characterized by the presence of abdominal stripes and gill rakers more than 37. *P. polyuranodon* differs from the others in having a large median vomerine tooth patch, very small palatine tooth patch and maxillary barbels extending posteriorly to gill opening.

B. The family Schilbeidae

Thai schilbeids were classified by the combination of numbers of barbels, the shape of cleft of mouth and palatal teeth. Three genera with four species were recognized. The genus *Eutropiichthys* with a single species, *E. salweenensis* (Figure 5A) differs from other schilbeids in having four pairs of barbels, one nasal, one maxillary and two mandibular pairs. The cleft of the mouth is oblique, extending to the front border of the eyes. The genus *Clupisoma* also possesses four pairs of barbels but the cleft of the mouth is not oblique, not extending to front edge of the eyes. There are two species, *C. sinense* (Figure 5B) with small ovoid and oblique palatal tooth patches and *C. prateri* (Figure 5C) with two separate elongated palatal tooth patches. *Laides* can be distinguished from other schilbeids in having three pairs of

barbels (nasal barbels absent). It contains a single species of *L. longibarbis* (Figure 5D).

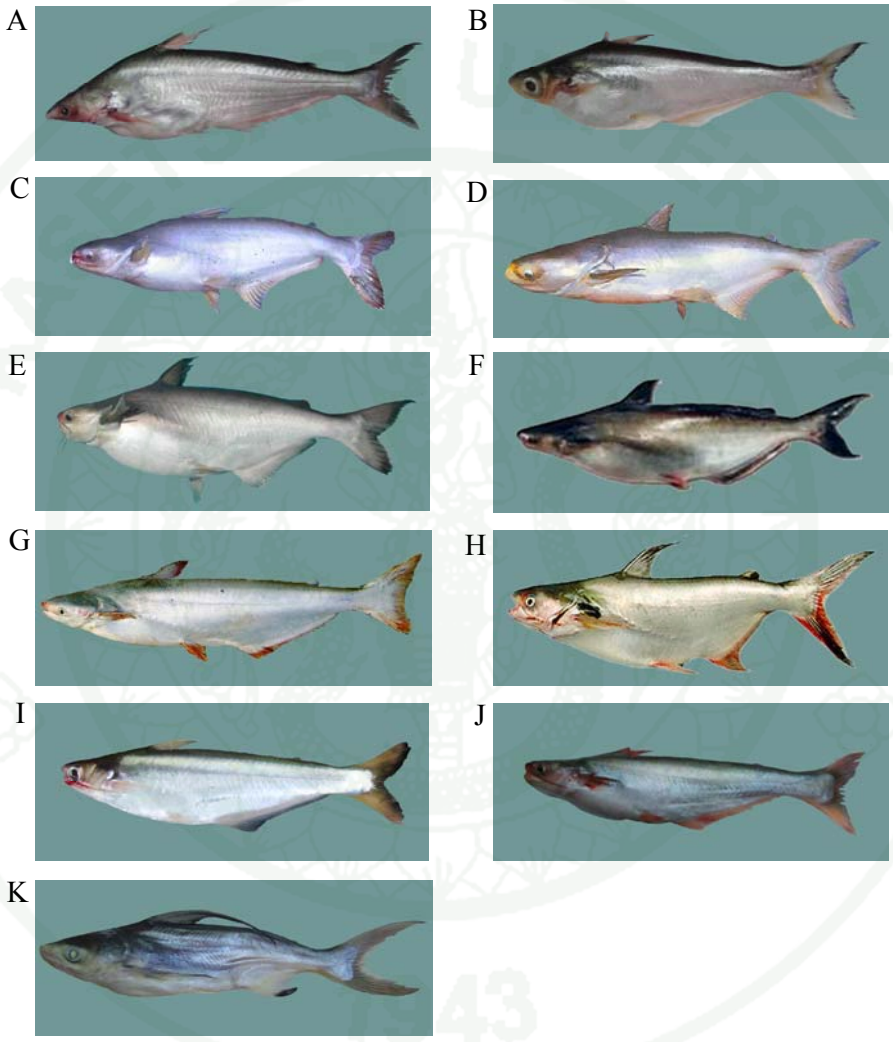


Figure 4 The extant pangasiid species in Thailand: (A) *Helicophagus leptorhynchus*, (B) *Pseudolais pleurotaenia*, (C) *Pangasianodon hypophthalmus*, (D) *P. gigas*, (E) *Pangasius bocourti*, (F) *P. conchophilus*, (G) *P. krempfi*, (H) *P. larnaudii*, (I) *P. macronema*, (J) *P. polyuranodon* and (K) *P. sanitwongsei.*

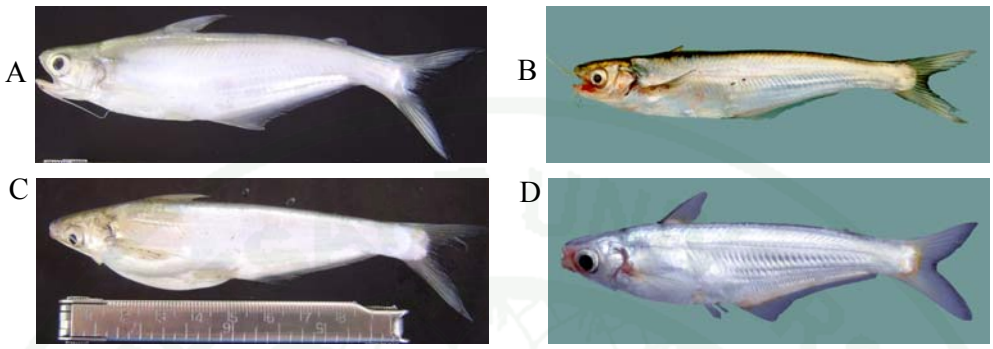


Figure 5 The extant schilbeid species in Thailand
(A) *Eutropiichthys salweenensis*
(B) *Clupisoma sinense*
(C) *Clupisoma prateri*
(D) *Laides longibarbis*

2. PCR amplification and sequence characteristics

A. *Cyt b* gene

The *cyt b* fragment was amplified using a combination of primer L15058 (Kocher *et al*., 1989) and either primer H16249 (Kocher *et al*., 1989) for schilbeid samples or primer DonThrR (San Mauro *et al*., 2004) for pangasiid samples. The approximately 1,140 bp of *cyt b*'s PCR products were obtained from all pangasiid and schilbeid specimens (Figure 6).

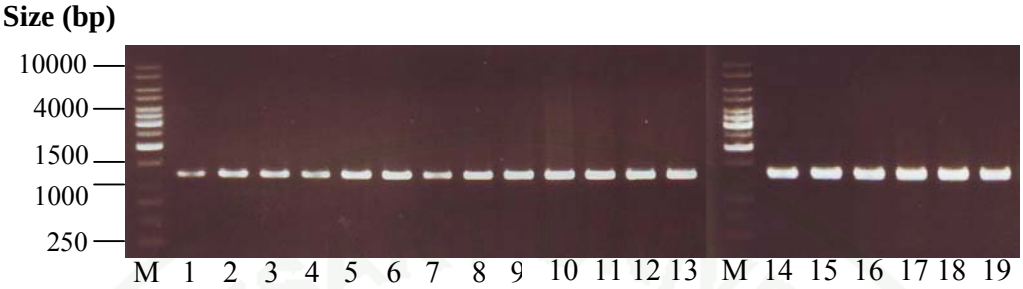


Figure 6 *Cyt b* gene's PCR products of all 19 pangasiid and schilbeid species. Each lane indicates the yield of DNA band as follow: M = the size of standard marker (Gene Ruler 1 kb DNA Ladder, Fermentas), 1 = *Pangasius bocourti*, 2 = *P.conchophilus*, 3 = *P. krempfi*, 4 = *P. larnaudii*, 5 = *P. macronema*, 6 = *P. nasutus*, 7 = *P. polyuranodon*, 8 = *P. sanitwongsei*, 9 = *Pseudolais pleurotaenia*, 10 = *Pangasianodon hypophthalmus*, 11 = *P. gigas*, 12 = *Helicophagus leptorhynchus*, 13 = *H. typus*, 14 = *C. sinense*, 15 = *C. prateri*, 16 = *Laides longibarbis*, 17 = *E. salweenensis*, 18 = *Pseudeutropius brachypopterus*, 19 = *P. moolenburghae*.

Nucleotide sequencing length of the *cyt b* fragments ranged from 1,106 to 1,140 bp. All new 35 sequences were determined through BLAST searches against GenBank (http://blast.ncbi.nlm.nih.gov/blast.cgi) and were 92-99 % similarity compared to other *cyt b* fragments deposited in GenBank database. Thirty-five *cyt b* sequences of pangasiids and schilbeids along with two sequences from *Helicophagus waandersii* (DQ119468; Hardman, 2005) and *Laides hexanema* (EU490915) and one outgroup sequence, *Ictalurus punctatus* (AF482987; Waldbieser, 2003) were then aligned. After complete alignments, the excessive nucleotides of two flank sides were trimmed. Then the final *cyt b* alignment was recorded as 1,106 bp and there is no gap throughout this gene. Of the 1,106 characters sampled across all taxa, 656 (59%) characters were constant and 450 (41%) characters were variable (417 (38%) variable parsimony informative and 33 (3%) variable parsimony uninformative sites). Of all informative characters, 75% came from the third codon position, and 21% and 4% from first and second positions, respectively (Table 7). This result indicated that the number of nucleotide substitution is highest for the third position, reflecting the fact

that many synonymous substitutions may occur at this codon position (Nei and Kumar, 2000).

Among 25 pangasiid individuals belonging to 13 species, a total of 21 *cyt b* different haplotypes were distinguished. Within schilbeids, a total of 8 *cyt b* haplotypes were found among 10 individuals of six species. For this genetic locus, the shared haplotypes were not determined between different species. All new sequences have been deposited at GenBank under accession numbers (GQ856790-GQ856796, HM236378-236386, HM236390-HM236401 and HM355762) as shown in Table 2.

B. RNA data set

To get the contiguous fragment of the posterior 3'end of the *12S rRNA* gene, $tRNA^{Val}$ gene and the 5' end of the *16S rRNA* gene (approximate length 2,000 bp), three DNA segments of approximately 390, 1,290 as well as 550 bp (Figure 7) were amplified and sequenced. By using BLAST searches against GenBank, the identities of the newly amplified sequences were determined with 89-98 % similarity compared with *12S rRNA*, *16S rRNA* and $tRNA^{Val}$ sequences deposited in GenBank. Three overlapping sequences were assembled and the total length ranged from 1,850 to 2,093 bp. Then thirty-five contiguous sequences of all pangasiids and schilbeids were aligned along with one sequence of *I. punctatus* (AF482987; Waldbieser, 2003) as outgroup. After the gap exclusion, the data set was remained 1,850 bp. Of the 1,850 characters sampled across all taxa, 1,495 (81%) characters were constant and 355 (19%) were variable (289 (16%) variable informative and 66 (3%) variable uninformative sites) (Table 7).

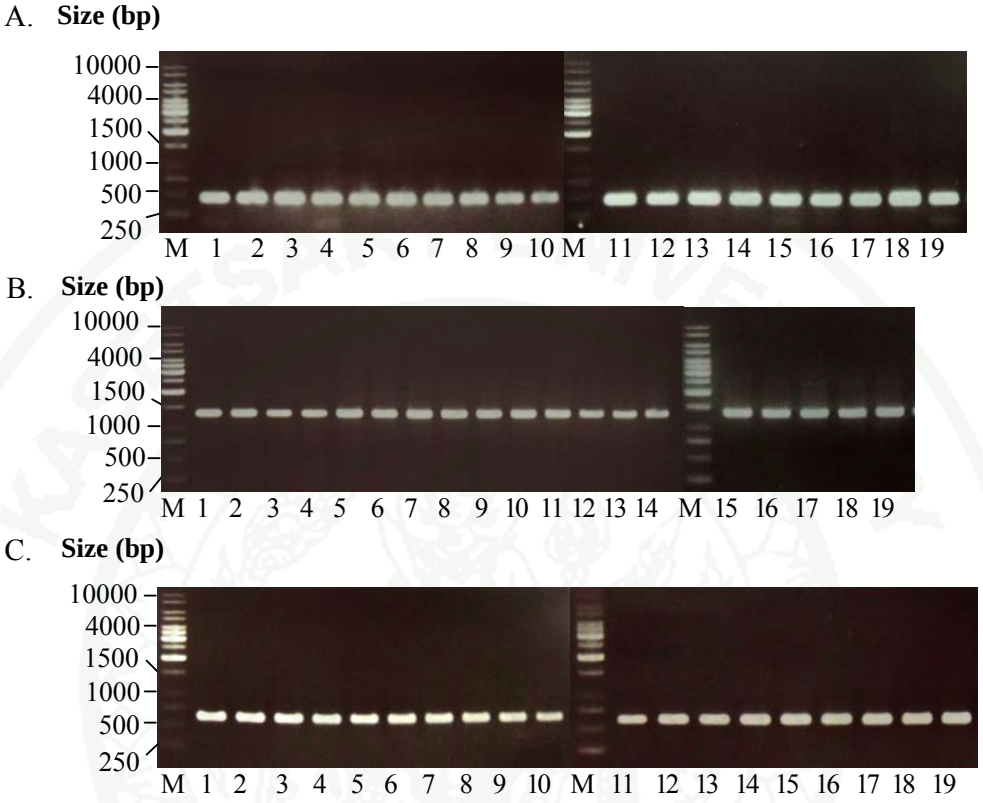


Figure 7 Three overlapping PCR fragments of RNA segment: A = approximately 390 bp of *12S rRNA*, B = approximately 1,290 bp of 3'-end of *12S rRNA*, $tRNA^{Val}$ and 5'-end of *16S rRNA* and C = approximately 550 bp of *16S rRNA*. Each lane indicates the yield of DNA band as follow: M = the size of standard marker (Gene Ruler 1 kb DNA Ladder, Fermentas), 1 = *Pangasius bocourti*, 2 = *P.conchophilus*, 3 = *P. krempfi*, 4 = *P. larnaudii*, 5 = *P. macronema*, 6 = *P. nasutus*, 7 = *P. polyuranodon*, 8 = *P. sanitwongsei*, 9 = *Pseudolais pleurotaenia*, 10 = *Pangasianodon hypophthalmus*, 11 = *P. gigas*, 12 = *Helicophagus leptorhynchus*, 13 = *H. typus*, 14 = *C. sinense*, 15 = *C. prateri*, 16 = *Laides longibarbis*, 17 = *E. salweenensis*, 18 = *Pseudeutropius brachypopterus*, 19 = *P. moolenburghae*.

Among 25 pangasiid individuals belonging to 13 species, a total of 18 different haplotypes for RNA data set were distinguished, while among 10 individuals of six schilbeid species, a total of 10 haplotypes were found. For this locus, the shared

haplotypes were not determined between different species. All new sequences have been deposited at GenBank under accession numbers (HM355752-HM355761 and HM355763-HM355780) as shown in Table 2.

C. *RAG1* gene

The *RAG1* PCR products were approximately 909 bp from all pangasiid and schilbeid specimens (Figure 8). Nucleotide sequencing of the partial *RAG1* fragments ranged in length from 858 to 909 bp. All new 35 sequences were determined through BLAST searches against GenBank and the 90-98 % similarities compared with *RAG1* sequences deposited in GenBank were obtained. Thirty-five *RAG1* sequences of pangasiids and schilbeids then were aligned along with an outgroup sequence of *I. punctatus* (AF482987; Waldbieser, 2003). The excessive nucleotides of two flank sides from each sequence were trimmed. There was no gap throughout this gene and the final *RAG1* alignment was recorded as 858 bp. Of the 858 characters sampled across all taxa, 697 (81%) characters were constant and 161 (19%) characters were variable (131 (15%) variable parsimony informative and 30 (4%) variable parsimony uninformative). Of all informative characters, 70% came from the third codon position, and 22% and 8% from the first and the second positions, respectively (Table 7).

Among 25 pangasiid individuals belonging to 13 species, a total of 15 different *RAG1* haplotypes were distinguished. Nine haplotypes were found among 10 individuals of six schilbeid species. For this locus, the shared haplotypes were not determined between different species. All new sequences have been deposited at GenBank under accession numbers (HM355781-HM355804) as shown in Table 2.

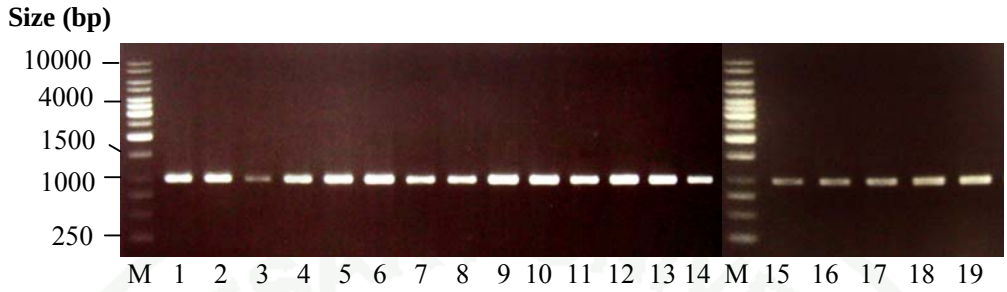


Figure 8 *RAG1* gene's PCR products of all 19 pangasiid and schilbeid species. Each lane indicates the yield of DNA band as follow: M = the size of standard marker (Gene Ruler 1 kb DNA Ladder; Fermentas), 1 = *Pangasius bocourti*, 2 = *P.conchophilus*, 3 = *P. krempfi*, 4 = *P. larnaudii*, 5 = *P. macronema*, 6 = *P. nasutus*, 7 = *P. polyuranodon*, 8 = *P. sanitwongsei*, 9 = *Pseudolais pleurotaenia*, 10 = *Pangasianodon hypophthalmus*, 11 = *P. gigas*, 12 = *Helicophagus leptorhynchus*, 13 = *H. typus*, 14 = *C. sinense*, 15 = *C. prateri*, 16 = *Laides longibarbis*, 17 = *E. salweenensis*, 18 = *Pseudeutropius brachypopterus*, 19 = *P. moolenburghae*.

Table 7 Properties of character variation for *cyt b*, RNA and *RAG1* data sets

Data set	Total sites	Constant sites	Variable sites	
			Informative	Uninformative
Cyt b	1,106	656	417	33
1st position	369	271	87	11
2nd position	368	344	17	7
3rd position	369	41	313	15
RNA	1,850	1,495	289	66
RAG1	858	697	131	30
1st position	286	251	29	6
2nd position	286	274	11	1
3rd position	286	172	91	23

3. Base composition and Chi-square test of homogeneity of base frequencies

A. *Cyt b* gene

Base composition of each of the four bases was determined for all codon positions and was calculated by averaging base composition values from individuals of each species. The mean base composition of each pangasiid and schilbeid species are shown in Table 8. Mean base composition of 14 pangasiid species (13 from this study and one from previous study (*Helicophagus waandersii*: DQ119468; Hardman, 2005) was determined as A = 27.92%, T = 28.63%, C = 29.81% and G = 13.64%. Similar contents were also found in schilbeids (six species from this study and one from previous study; *Laides hexanema*, EU490915) as A = 29.44%, T = 28.99%, C = 27.69% and G = 13.88%. This result revealed a moderately antiguanine bias. The lower value was largely due to the instability of G content at the third codon position. A strong selection against the use of guanine at third codon positions of protein-coding genes is a typical feature of vertebrate mtDNA (Zardoya and Meyer, 2000) and is similar to those previously reported for Actinopterygian fish (Peng *et al*., 2004) such as sisorid catfishes (Sisoridae: A = 29.30%, T = 28.10%, C = 29.20% and G = 13.40%; Peng *et al*., 2004) and percid fishes (Percidae: A = 22.70%, T = 30.20%, C = 30.70% and G = 16.40%; Sloss *et al*., 2004).

Table 8 Percentage of base composition and a chi-square test of base homogeneity for all codon positions of the *cyt b* data set

Species	Nucleotide Bases				Total sites
	T	C	A	G	
Pangasius bocourti	28.70	29.50	28.50	13.30	1106
Pangasius conchophilus	28.60	30.10	28.00	13.30	1106
Pangasius krempfi	29.20	29.20	27.15	14.45	1106
Pangasius larnaudii	28.15	30.05	27.95	13.85	1106
Pangasius macronema	28.70	29.50	28.40	13.40	1106
Pangasius nasutus	28.80	29.85	27.85	13.50	1106

Table 8 (Continued)

Species	Nucleotide Bases				Total sites
	T	C	A	G	
Pangasius polyuranodon	28.80	29.30	28.60	13.30	1106
Pangasius sanitwongsei	28.60	29.70	28.00	13.70	1106
Pseudolais pleurotaenia	29.10	29.50	27.80	13.60	1106
Pangasianodon hypophthalmus	29.10	29.20	28.20	13.50	1106
Pangasianodon gigas	28.30	30.50	27.30	13.90	1106
Helicophagus leptorhynchus	28.10	30.50	27.60	13.80	1106
Helicophagus typus	28.40	30.20	27.80	13.60	1106
Helicophagus waandersii	28.20	30.30	27.80	13.70	1106
Clupisoma sinense	27.90	28.80	28.90	14.40	1106
Clupisoma prateri	29.50	27.00	29.80	13.70	1106
Laides longibarbis	28.40	27.80	29.90	13.90	1106
Laides hexanema	28.50	27.70	30.00	13.80	1106
Eutropiichthys salweenensis	29.95	26.60	29.25	14.20	1106
Pseudeutropius brachypopterus	28.70	28.60	28.90	13.80	1106
Pseudeutropius moolenburghae	30.00	27.30	29.30	13.40	1106
Ictalurus punctatus	28.50	30.70	26.20	14.60	1106
Average	28.80	29.20	28.30	13.70	1106
χ^2			39.17		
d.f.			111		
***p*-value**			1.00		

Changes in nucleotide frequency among different lineages in a data set are thought to lead to erroneous phylogenetic inference because unrelated clades may appear similar because of evolutionarily unrelated similarities in nucleotide frequencies (Page and Holmes, 1998). To dictate the potentially misleading effects of heterogeneous base composition among all taxa in phylogenetic reconstruction, the chi-square test of homogeneity of base frequencies across taxa was implemented in PAUP* with BASEFREQS command. At the $p < 0.05$ level, there was no statistically significant proportion differences among all taxa ($\chi^2 = 39.17$, d.f.= 111, $p = 1.00$), indicating that the base composition among surveyed taxa in *cyt b* data set is

stationary across pangasiids, schilbeids and outgroup species. It presumably did not greatly influence the phylogenetic analyses (Orrell and Carpenter, 2004).

B. RNA data set

Base composition of each of the four bases was calculated by averaging base composition values from individuals of each species (13 pangasiids and six schilbeids from this study). The mean base composition of each pangasiid and schilbeid species are shown in Table 9. Mean values of percent base composition for all positions of the RNA data set estimating in pangasiids were determined as follow: A = 31.83%, C = 24.69%, G = 21.22% and T = 21.3%. The equivalent values were also found in schilbeids as A = 32.94%, C = 23.84%, G = 21.78% and T = 21.44%. This result revealed that there was bias towards the use of adenine and this feature of mt *rRNA* genes was similar to that reported in other fishes such as gobioid fishes (Gobioidei: A = 30.30%, C = 25.60, G = 22.90, T = 21.20%; Wang and Lee, 2002), doradid catfishes (Doradidae: A = 31.41%, C = 25.96, G = 21.48, T = 21.15; Moyer *et al*., 2004) and butterfilyfishes (Chaetodontidae: A = 30.10%, C = 25.30%, G = 22.90, T = 21.60%; Fessler *et al*., 2007).

Chi-square tests of homogeneous base frequencies among taxa for all positions (χ^2 =15.71, df=105 p=1.00) (Table 9) failed to reject the null hypothesis (at the $P < 0.05$ level) which is an implication that the base composition among surveyed taxa in the RNA data set is stationary across Pangasiidae, Schilbeidae as well as outgroup species and this should not cause the misconstruction of phylogeny (Orrell, 2000).

Table 9 Percentage of base composition and a chi-square test of base homogeneity for all positions of the RNA data set

Species	All positions				
	T	**C**	**A**	**G**	**Total**
Pangasius bocourti	21.30	24.50	31.70	22.50	1850
Pangasius macronema	21.35	24.35	31.95	22.35	1850
Pangasius polyuranodon	20.90	24.80	31.90	22.40	1850
Pangasius conchophilus	21.20	24.60	32.00	22.20	1850
Pangasius krempfi	21.00	25.00	31.60	22.40	1850
Pangasius larnaudii	21.35	24.45	31.70	22.50	1850
Pangasius nasutus	21.20	24.80	32.10	21.90	1850
Pangasius sanitwongsei	21.10	24.90	31.70	22.30	1850
Pseudolais pleurotaenia	21.10	24.80	32.00	22.10	1850
Pangasianodon hypophthalmus	21.90	24.20	31.70	22.20	1850
Pangasianodon gigas	21.30	24.60	31.90	22.20	1850
Helicophagus leptorhynchus	21.10	25.00	31.70	22.20	1850
Helicophagus typus	21.10	25.00	31.70	22.20	1850
Pseudeutropius moolenburghae	21.70	23.70	32.50	22.10	1850
Pseudeutropius brachypopterus	21.70	23.80	32.60	21.90	1850
Laides longibarbis	21.40	23.70	33.20	21.70	1850
Clupisoma prateri	21.15	24.25	33.10	21.50	1850
Clupisoma sinense	21.50	23.70	33.05	21.75	1850
Eutropiichthys salweenensis	21.20	23.90	33.20	21.70	1850
Ictalurus punctatus	21.40	24.50	32.20	21.90	1850
Average	21.30	24.50	32.20	22.00	1850
χ^2	15.71				
d.f.	105				
***p*-value**	1.00				

C. *RAG1* gene

Base composition of each of the four bases of *RAG1* sequence was determined for all codon positions and was calculated by averaging base composition values from individuals of each species (13 pangasiids and six schilbeids). The overall base compositions for *RAG1* data set within pangasiids were determined as follow: A = 25.75%, C = 21.60%, G = 27.04% and T = 25.61%. This fairly uniform of base composition was also found in schilbeids as A = 26.11%, C = 21.51%, G = 26.73% and T = 25.65%. The mean base composition of each pangasiid and schilbeid species are shown in Table 10. According to the result obtained from this study, the nuclear *RAG1* sequence was homogenous in overall base composition which is similar to what has been reported for this gene in other fishes such as damselfishes (Pomacentridae: A = 25%, C = 29%, G = 24%, T = 22%; Quenouille *et al*., 2004), cyprinid fishes (Cyprinidae: A = 25.70%, C = 24.05%, G = 27.02%, T = 23.23%; Schönhuth *et al*., 2008) and North American phoxinins (Leuciscidae: : A = 25.88%, C = 24.00%, G = 26.73%, T = 23.39%; Bufalino and Mayden, 2010).

Chi-square tests of homogeneous base frequencies among taxa for all codon positions ($\chi^2 = 3.82$, df = 105, $p = 1.00$) (Table 10) failed to reject the null hypothesis (at the $P < 0.05$ level), which is an implication that the base composition among surveyed taxa in *RAG1* data set is stationary across Pangasiidae, Schilbeidae and outgroup species and this should not distort phylogenetic inference (Orrell, 2000).

Table 10 Percentage of base composition and a chi-square test of base homogeneity for all codon positions of the *RAG1* data set.

Species	Nucleotide Bases				Total sites
	T	C	A	G	
Pangasius bocourti	25.35	21.65	26.00	27.00	858
Pangasius conchophilus	25.60	21.60	25.50	27.30	858
Pangasius krempfi	25.90	21.50	25.80	26.80	858
Pangasius larnaudii	25.30	21.70	25.60	27.40	858
Pangasius macronema	26.00	21.60	25.40	27.00	858
Pangasius nasutus	25.50	21.70	25.50	27.30	858
Pangasius polyuranodon	26.00	21.60	25.40	27.00	858
Pangasius sanitwongsei	25.50	21.70	25.50	27.30	858
Pseudolais pleurotaenia	25.60	21.70	25.40	27.30	858
Pangasianodon hypophthalmus	25.20	21.80	26.00	27.00	858
Pangasianodon gigas	25.60	21.50	25.90	27.00	858
Helicophagus leptorhynchus	25.75	21.35	26.30	26.60	858
Helicophagus typus	25.60	21.40	26.40	26.60	858
Clupisoma sinense	25.75	21.55	25.75	26.95	858
Clupisoma prateri	25.70	21.70	26.10	26.50	858
Laides longibarbis	25.85	21.30	25.50	27.35	858
Eutropiichthys salweenensis	25.70	21.40	25.80	27.10	858
Pseudeutropius brachypopterus	25.40	21.40	26.70	26.50	858
Pseudeutropius moolenburghae	25.50	21.70	26.80	26.00	858
Ictalurus punctatus	26.10	20.90	26.10	26.90	858
Average	25.60	21.60	25.80	27.00	858
χ^2	3.82				
d.f.	105				
***p*-value**	1.00				

4. Incongruence Length Difference (ILD) test for combined data set

In this study, three genetic loci including *cyt b* (1,106 bp), RNA (1,850 bp) and *RAG1* (858 bp) genes were incorporated into a combined data set for phylogenetic reconstruction. However, prior to the combination of all data sets, the ILD test (Farris *et al*., 1995) was used to examine possible incongruence between different genes (*cyt b* vs. RNA, *cyt b* vs. *RAG1*, RNA vs. *RAG1* and mtDNA vs. nuDNA) by means of the partition homogeneity test in PAUP* v. 4.0b10 (Swofford, 2002). The results indicated that the partition homogeneity test detected a significant congruence (at the $p < 0.05$ level) between all data partitions as follow: *cyt b* vs. RNA, $p = 0.66$; *cyt b* vs. *RAG1*, $p = 1.00$; RNA vs. *RAG1*, $p = 0.22$; and mtDNA vs. nuDNA, $p = 0.84$. As there was no evidence for a phylogenetic conflict among the different loci ($p > 0.05$), thus all nucleotide data sets were combined into a single data set for further phylogenetic analyses.

After combining all nucleotide sequences, the concatenated data set of all 35 pangasiid and schilbeid samples along with one outgroup species, *I. punctatus* (Accession number: AF482987; Waldbieser, 2003) consisted of 3,814 bp. Of these 3,814 characters, 2,848 characters (74.67%) were constant and 966 characters (25.33%) were variable (837 (21.95%) variable parsimony informatives and 129 (3.38%) variable parsimony uninformatives).

5. The best-fit evolutionary model for each nucleotide data set

The best evolutionary model of nucleotide substitution that best fitted for each nucleotide data set was chosen by the program Modeltest v. 3.7 (Posada and Crandall, 1998), based on the Akaike Information Criterion (AIC).

The best evolutionary model for the cyt *b* data set was the GTR + I + G (General Time Reversible model + I + G) model (Rodriquez *et al*., 1990), with a proportion of invariable sites (I) of 0.5527 and a gamma distribution (G) with a shape parameter $\alpha = 1.6030$.

The best evolutionary model for the RNA data set was also the GTR + I + G model (Rodriquez *et al*., 1990), with a proportion of invariable sites (I) of 0.5998 and a gamma distribution (G) with a shape parameter α = 0.4682.

The best evolutionary model for the RAG1 data set was the HKY + I + G (Hasegawa-Kishino-Yano model + I + G) (Hasegawa *et al*., 1985), with a proportion of invariable sites (I) of 0.5004 and a gamma distribution (G) with a shape parameter α = 0.9604

The evolutionary model for each data set was further used to estimate genetic distances and to reconstruct phylogeny in NJ, ML and BI analyses.

Since the resulting ILD test indicated a significant congruence between three genetic loci and thus all data set were combined for phylogenetic analysis. The best evolutionary model for the combined data was the GTR + I + G model (Rodriquez *et al*., 1990), with a proportion of invariable sites (I) of 0.5109 and a gamma distribution (G) with a shape parameter α = 0.4195 (GTR+ I + G). The obtained model for the combined data set would be used to reconstruct NJ and ML trees. For BI analysis, models of sequence evolution for different partitions of sequence data set (*cyt b*, RNA and *RAG1*) were allowed to separately implement.

6. Substitution saturation test

Nucleotide saturation analysis was performed to test the occurrence of multiple substitution by plotting the total number of nucleotide differences (Ts, Tv and Ts+Tv) on Y axis against the pairwise genetic distances which were calculated based on the best-fit evolutionary model on X axis.

A. *Cyt b* gene

Plots of total number of Ts, Tv and Ts+Tv (Y-axis) versus pairwise genetic distances (X-axis) based on the GTR + I + G model demonstrated that the

number of nucleotide difference does not increase linearly as a function of genetic distance at distance greater than 0.3 (Figure 9) which corresponds to the pairwise distance between genera of pangasiids and schilbeids. This result indicates some levels of nucleotide saturation in *cyt b* data set.

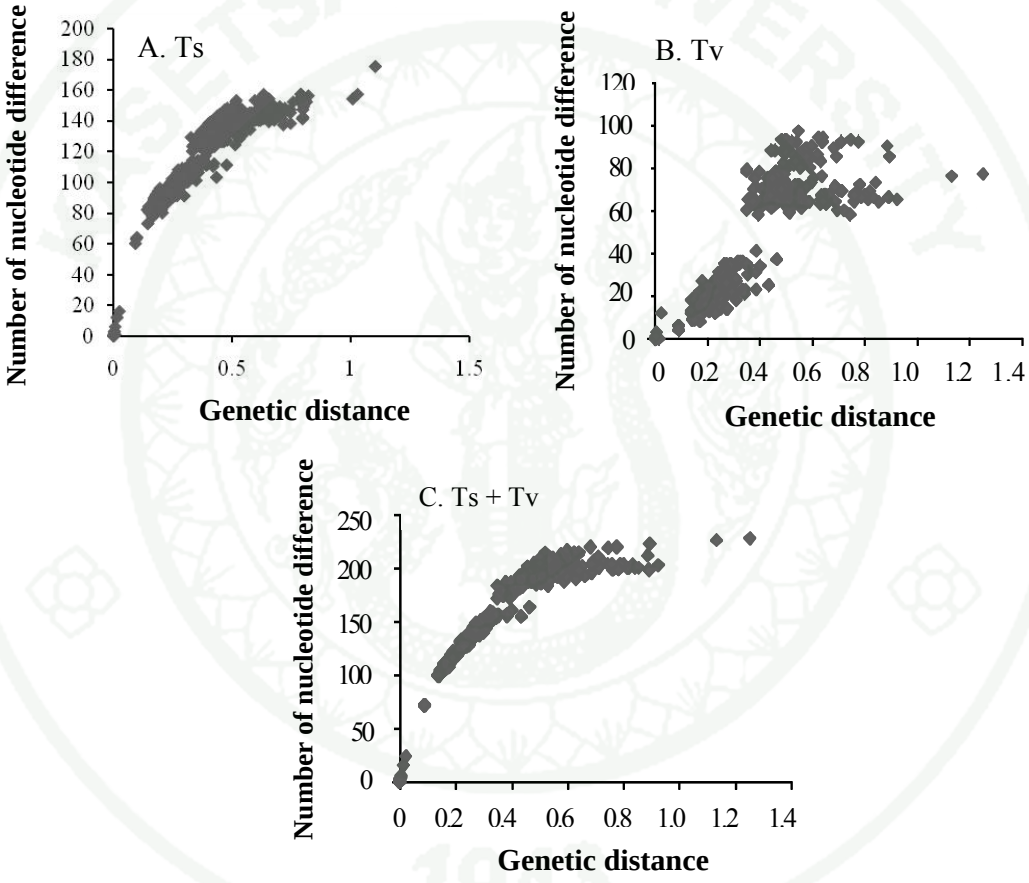


Figure 9 Plots showing the number of nucleotide difference: A = Ts, B = Tv and C = Ts + Tv versus pairwise genetic distance based on *cyt b* sequences within pangasiid and schilbeid specimens and outgroup species.

Often, third codon positions are assumed to be saturated and devoid of phylogenetic information and therefore, they should be excluded from analysis (Zharkikh and Li, 1992). However, in this study, when the third codon positions were excluded from the phylogenetic analyses, the statistical confidences at generic and

species levels were reduced (Appendix Figure 1-4). In ML (Appendix Figure 3) and BI (Appendix Figure 4) trees, the topologies are similar to the trees analysed from all codon positions but have an underestimate of statistical support at almost all the interior branches. Less resolved phylogenies are observed in NJ (Appendix Figure 1) and MP (Appendix Figure 2) analyses in which non-monophyletic assemblage of schilbeids is found with the separation of *Pseudeutropius*. Moreover, in MP analysis, the intrarelationships within pangasiids are unresolved since the polytomy branches occur among them. In all analyses with the exclusion of *cyt b*'s third codon positions, the intrarelationships of *Pangasius* and *Helicophagus* are also unresolved. Unresolved and/or poorly support phylogeny derived from the analysis of *cyt b* sequences without the third codon positions was also found in the other studies. Farias *et al*. (2001) who proposed the *cyt b* phylogeny of Cichlidae revealed that saturation at third codon positions of *cyt b* sequences initiated at the *p*-distance close to 0.15, indicating that saturation might be a problem at the intergeneric level. Although the third positions were excluded from the analyses, the topologies changed to an unreasonable grouping of certain cichlid lineages and revealed several polytomies. Similar result was also found in the stromateoid fish phylogeny. Doiuchi and Nakabo, 2006 found the multiple substitusion at the third codon positions of *cyt b* at the intergeneric level (beyond 0.2 Kimura's two parameter distance) but the removal of the third codon positions in analyses resulted in less supported phylogenies, especially at the generic level. Moreover, a less resolved phylogeny also found in cobitid fish phylogeny in which the third codon position in the *cyt b* data set were excluded (Šlechtová *et al*., 2008). Håstad and Björklund (1998) indicated that the effect of the removal of the third codon positions is to remove most of the information at the same time. These sites contain important signal which was informative at least for closely related species. Consequently, it was better to use all sites of *cyt b* sequence for inferring phylogenetic relationships in this study.

B. RNA data set

Plots of total number of Ts, Tv and Ts+Tv (Y-axis) versus pairwise genetic distances (X-axis) based on the GTR + I + G model for the RNA data set

revealed linear relationships (Figure 10) which indicates that this data set was not saturated (i.e. a little or no additional substitution is detectable with increased genetic distance). Consequently, this nucleotide data set could be further used to estimate the phylogenetic relationships (Salemi and Vandamme, 2003) of pangasiids and schilbeids.

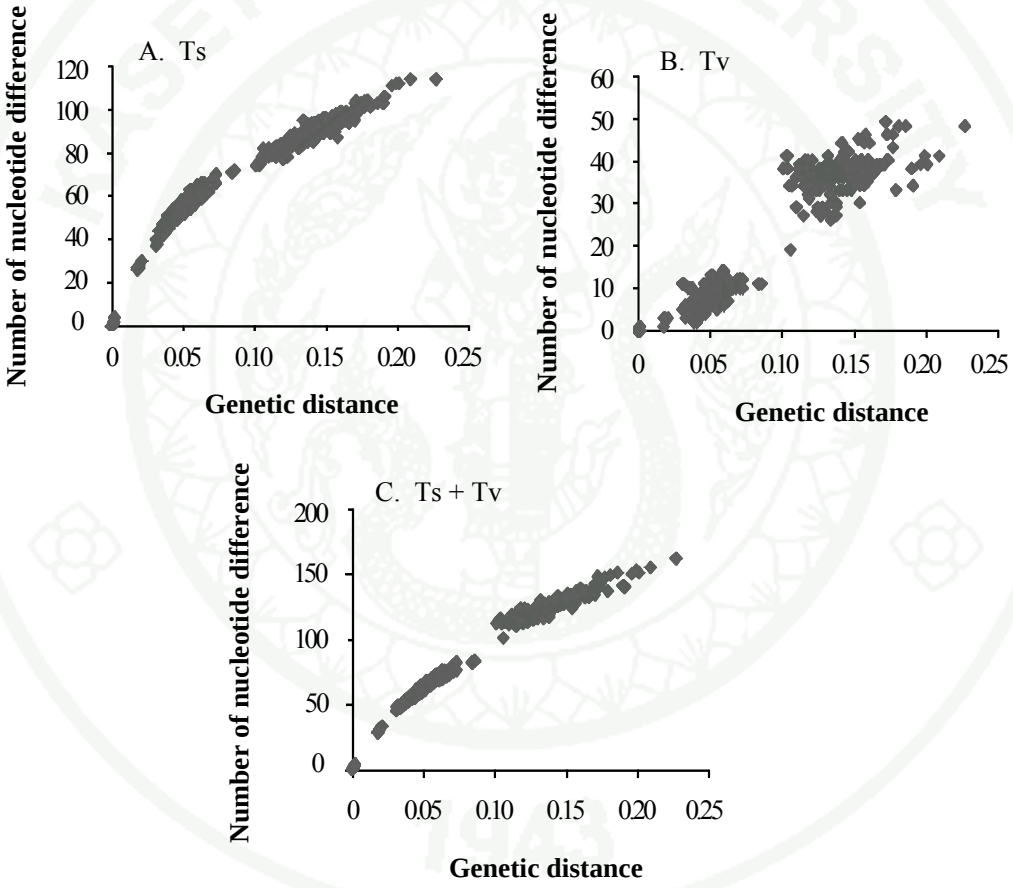


Figure 10 Plots showing the number of nucleotide difference: A = Ts, B = Tv and C = Ts + Tv versus pairwise genetic distance based on RNA sequences within pangasiid and schilbeid specimens and outgroup species.

C. *RAG1* gene

Plots of total number of Ts, Tv and Ts+Tv (Y-axis) against pairwise evolutionary distance (X-axis) which were determined based on the HKY + I + G evolutionary model indicated an absence of nucleotide saturation. Linear relationship (Figure 11) which was found in all plots means that the number of transitional (Ts), transversional (Tv) as well as the number of Ts+Tv diferrences from pairwise comparisons increased with increasing of the evolutionary distance. Because no such plateau is seen, thus all positions of *RAG1* nucleotide sequences could be further used to determine the phylogenetic relationships among Pangasiidae and Schilbeidae.

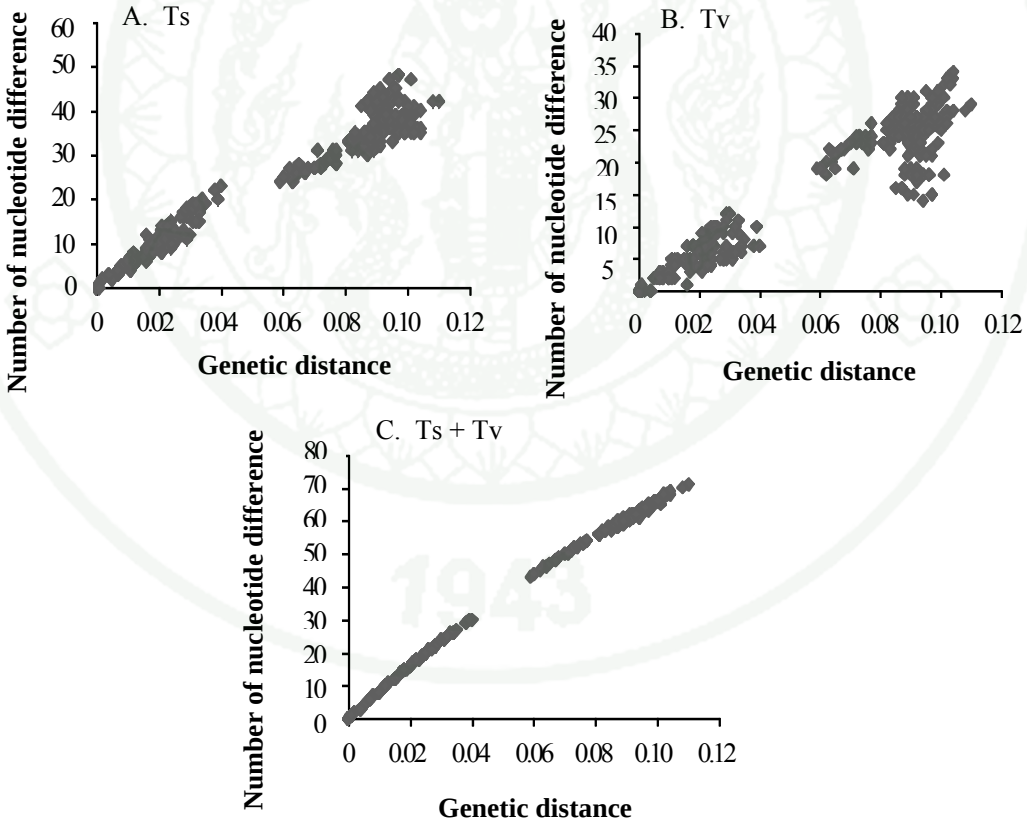


Figure 11 Plots showing the number of nucleotide difference: A = Ts, B = Tv and C = Ts + Tv versus pairwise genetic distance based on *RAG1* sequences within pangasiid and schilbeid specimens and outgroup species.

7. Nucleotide sequence divergences

Sequence divergence or *p*-distance is the proportion of different homologous sites which is expressed as the number of nucleotide differences per site (Strimmer and von Haeseler, 2003). Several studies used the *p*-distance values as the criterion to indicate taxonomic ranks at various levels (family, genus and species) of fishes (Johns and Avise, 1998; Doadrio and Domínguez, 2004; Heyden and Matthee, 2008; Rocha *et al*., 2008; Doadrio *et al*., 2009). In the present study, the *p*-distance values for each genetic marker were determined by using MEGA v. 4.0 (Kumar *et al*., 2008). The values were compared with previously published papers (e.g. Ritchie *et al*., 1996; Johns and Avise, 1998; Doadrio and Domínguez, 2004; Miller and Cribb, 2007; Almada *et al*., 2008; Heyden and Matthee, 2008; Rocha *et al*., 2008; Doadrio *et al*., 2009) and were used as the criterion to delimit taxonomic ranks (familial, generic and specific levels) of pangasiid and schilbeid catfishes.

A. *Cyt b* gene

For the *cyt b* gene, the values of the interfamilial *p*-distance among the families of the Order Siluriformes were reported as a mean of 19.81% (Kartavtsev *et al*. 2007) and among the families of the Order Gadiformes were determined as 16% - 27% (Heyden and Matthee, 2008). At the generic level, John and Avise (1998) reported genetic distances for confamilial genera of fishes ranged from 8% to 25%, with an average of 14%. The mean intergeneric variation estimated from the four catfish families was 16.37% (Kartavtsev *et al*., 2007). The corresponding values reported in fishes of order Cyprinidontiformes within Poeciliidae (Doadrio *et al*., 2009) and Goodeidae (Doadrio and Domínguez, 2004), ranged from 8% to 11%. In addition, a mean sequence divergence among genera of botiid loaches (Botiidae) was estimated as 14.5% (Šlechtová *et al*., 2006). The *p*-distance value for separated species of the same genus (interspecific level) was previously reported with a wide range, 1.7-12.5%. Johns and Avise (1998) indicated the most frequent *cyt b* interspecific sequence divergence, estimating from 81 fish genera, was approximately 4.5%. The interspecific *p*-distance among *Noturus* catfishes ranged from 2.45% to

12.5% (Hardman, 2004). A similar value was also found within the families Goodeidae and Cyprinidae in which the interspecific *p*-distances were determeined as 1.7%-11% (Doadrio and Domínguez, 2004) and 2.1%-11.4% (Schönhuth *et al*., 2008), respectively. For the intraspecific level (among individuals of the same species), the variation was mostly reported as lower than 2.0%. The mean intraspecific *p*-distance value estimated from the four catfish families was 1.59% (Kartavtsev *et al*., 2007). Within the fish family Goodeidae, the intraspecific variation ranged from 0.01% to 1.7% (Doadrio and Domínguez, 2004) and within the cuban fishes genus *Girardinus* ranged from 0.09 to 1.1%; Doadrio *et al*., 2009).

In this study, 35 *cyt b* sequences of pangasiid and schilbeid specimens were used for *p*-distance estimation. Two additional sequences from GenBank, *Helicophagus waandersii* (DQ119468; Hardman, 2005) and *Laides hexanema* (EU490915) were also included to validate the putative new species status of *H. leptorhynchus* and *L. longibarbis* from this study. The resulting pairwise *p*-distances were averaged and were presented in the matrix of the interspecific (Table 11) and intergeneric *p*-distances (Table 12) of pangasiids and schilbeids.

The interfamilial *p*-distance values between pangasiid and schilbeid species ranged from 16.0% to 19.8% with a mean of 17.6%. These values are in the range of those previously reported at the interfamilial level among families within Siluriformes (19.81%; Kartavtsev *et al*. 2007) and within Gadiformes (16% - 27%; Heyden and Matthee, 2008). This result reconfirms that Pangasiidae and Schilbeidae are distinct families.

Within the family Pangasiidae, intergeneric *p*-distance ranged from 11.4% (*Pangasius* and *Pseudolais*) to 14.0% (*Pangasianodon* and *Helicophagus*) with a mean of 13.0% which is slightly lower than the average intergeneric *p*-distance estimated from the catfish families Amblycipididae, Bagridae, Ictaluridae and Siluridae (16.37%; Kartavtsev *et al*., 2007). However, the intergeneric *p*-distances among Pangasiidae obtained in this study were higher than the corresponding values reported in fishes of order Cyprinidontiformes within Poeciliidae (Doadrio *et al*.,

2009) and Goodeidae (Doadrio and Domínguez, 2004), which ranged from 8% to 11% but corresponded with a mean sequence divergence among genera of botiid loaches (Botiidae: 14.5%; Šlechtová *et al*., 2006). Moreover, a wider taxonomic survey reported genetic distances for confamilial genera of fishes ranged from 8% to 25%, with an average of 14% (Johns and Avise, 1998). In accordance with our result, it is suggested that the family Pangasiidae should be classified into four distinct genera: *Pangasius*, *Helicophagus*, *Pseudolais* and *Pangasianodon*.

At the interspecific level, the *p*-distance values between species for each pangasiid genus were reported as: *Pangasius*, 6.5%-12.3%; *Pangasianodon*, 10.2% and *Helicophagus*, 0.5%-1.4%. This is except for the genus *Pseudolais* in which a single species was included for the analysis. The interspecific variations presented in this study corresponded to *p*-distances used for separated species of the same genus in other fishes (1.7%-12.5%: Johns and Avise, 1998; Doadrio and Domínguez, 2004; Hardman, 2004; Schönhuth *et al*., 2008) as previously mentioned. Interestingly, a very low interspecific value (0.5%) was observed between *Helicophagus leptorhynchus* and *H. waandersii*. This value approaches or overlaps with values reported at the intraspecific level for fishes (Doadrio and Domínguez, 2004; Kartavtsev *et al*., 2007; Doadrio *et al*., 2009).

Regarding the intraspecific variation, there was no genetic difference between two individuals of five pangasiid species including *Pangasius polyuranodon*, *P. conchophilus*, *Pangasianodon hypophthalmus*, *P. gigas* and *Helicophagus leptorhynchus* (Table 13), whereas the intraspecific variation was found in seven species: *Pangasius bocourti* (1.2%), *P. macronema* (0.3%), *P. krempfi* (0.1%), *P. larnaudii* (0.5%), *P. sanitwongsei* (0.4%), *P. nasutus* (0.2%) and *Pseudolais pleurotaenia* (0.2%). These intraspecific variations were low and generally fell within reported ranges of within species *cyt b* divergence (0.01%-1.7%: Doadrio and Domínguez, 2004; Kartavtsev *et al*., 2007; Doadrio *et al*., 2009). This result confirms the validity of specimens for each recognized pangasiid species used in this study.

An extreamely low interspecific variation (0.5%) between *Helicophagus leptorhynchus* and *H. waandersii* was found in this study. Interestingly, this small value corresponded to the intraspecific *p*-distance found in *Pangasius larnaudii* (0.5%) and *P. sanitwongsei* (0.4%) from this study and less than the intraspecific *p*-distance found in other 15 catfish species (mean value = 1.59%; Kartavtsev *et al*., 2007). Based on morphological characteristics, *H. leptorhynchus* and *H. waandersii* are very similar and were previously recognized as *H. waandersii*. Although Ng and Kottelat (2000) described the Indochinese specimen to be a new pangasiid species as *H. leptorhynchus* which differs from *H. waandersii* from Sumatra and Peninsular Malaysia in having a longer anal fin, shorter caudal peduncle, longer head, larger eye and more slender snout. However, these morphometric characters can be mixed (Gustiano *et al*., 2004; Philasamorn and Satrawaha, 2009) and the variability may respect to growth of the specimens (Watanabe *et al*., 2007) which can lead to misidentification. The molecular evidence from this study suggests that *H. leptorhynchus* and *H. waandersii* should be the same species. Since *H. waandersii* Bleeker, 1858 was named before *H. leptorhynchus* (Ng and Kottelat, 1999), thus, *H. waandersii* is suggested as the valid species name.

Within the family Schilbeidae, the intergeneric *p*-distance extended from 10.6% (*Clupisoma* and *Laides*) to 17.1% (*Eutropiichthys* and *Pseudeutropius*) with a mean of 15.0%. These values are more elevated than those reported in Pangasiidae (mean = 13.0%) from this study and in fishes of order Cyprinidontiformes within Poeciliidae (Doadrio *et al*., 2009) and Goodeidae (Doadrio and Domínguez, 2004), which ranged from 8% to 11%. However, the intergeneric *p*-distances among Schilbeidae presented in this study fell within reported ranges of *cyt b* sequence divergences between fish genera (8%-25%) as described by Johns and Avise, (1998). Thus, the molecular evidence from this study confirms the existence of four genera of Schilbeidae including *Laides*, *Clupisoma*, *Eutropiichthys* and *Pseudeutropius*.

At the interspecific level, the *p*-distance values between species for each schilbeid genus were reported as: *Laides*, 2.7%; *Clupisoma*, 10.5% and *Pseudeutropius*, 14%. This is except for the genus *Eutropiichthys* in which a single

species was included for the analysis. The interspecific variations among Schilbeidae corresponded to those reported in Pangasiidae (0.5%-12.3%). The smallest interspecific variation (2.7%) was found between *Laides longibarbis* and *L. hexanema* which is native to Sumatra and Peninsular Malaysia. This value corresponded to the interspecific *p*-distances found in goodeid fishes (Goodeidae, 1.7%-11%; Doadrio and Domínguez, 2004) and southern North American cyprinids (Cyprinidae, 2.1%-11.4%; Schönhuth *et al*., 2008). *L. longibarbis* and *L. hexanema* were previously recognized as conspecific (Vidthayanon and Roongthongbaisuree, 1993) but subsequently were defined as two independent species by Ng, 1999. Based on the taxonomic revision of *L. hexanema* Ng (1999) found that the Indochinese specimens differ from the Sundaic specimens with the combination of a longer anal-fin base, a smaller eye and a larger interorbital distance. Thus, the Indochinese specimen was recognized to be distinct species as *L. longibarbis.* Based on the genetic difference, with 2.7% sequence divergence between *L. longibarbis* and *L. hexanema* observed in this study, the validity of the species status of *L. longibarbis* is confirmed. The next small interspecific *p*-distances of schilbeids (9.00%) was found between the enigmatic species, *Clupisoma sinense* and *L. longibarbis* and this value was less than the interspecific *p*-distance between *C. sinense* and its congener, *C. prateri* (10.5%). *C. sinense* was formerly recognized as *L. sinensis* (Rainboth, 1996), and there has been no molecular evidence to assess the taxonomic position of this species. The molecular data from this study may indicate that *C. sinense* and *L. longibarbis* most likely belong to the same genus, *Laides*, as described by Rainboth (1996).

At the intraspecific level, there was no genetic difference between two individuals of *L. longibarbis* and *C. prateri*, whereas the intraspecific variation was found in *C. sinensis* (0.4%) and *E. salweenensis* (0.2%). The intraspecific *p*-distance for *L. hexanema*, *P. brachypopterus* and *P. moolenburghae* were not obtained from this study because only one specimen was included for the analysis. The intraspecific variations within schilbeid species fell within reported ranges of *cyt b* divergence within fish species (0.01%-1.7%: Doadrio and Domínguez, 2004; Kartavtsev *et al*., 2007; Doadrio *et al*., 2009). The result confirmed the validity of specimens for each recognized pangasiid species used in this study.

Table 11 The values of *cyt b p*-distance (in percentage) between species of pangasiids and schilbeids (below diagonal) and within each species (diagonal values in bold font).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1) Pboc	**1.2**																
2) Pmac	11.4	**0.3**															
3) Ppol	10.9	6.5	**0.0**														
4) Pcon	10.2	9.0	9.2	**0.0**													
5) Pkre	10.5	9.9	9.8	9.0	**0.1**												
6) Plar	11.4	12.3	11.8	10.5	10.5	**0.5**											
7) Psan	10.4	9.9	10.2	9.2	9.5	10.4	**0.4**										
8) Pnas	10.2	10.5	10.1	6.5	9.9	11.4	9.2	**0.2**									
9) Pple	12.1	10.8	11.8	11.2	11.7	11.5	10.7	11.6	**0.2**								
10) Phyp	14.3	12.5	12.3	12.5	14.2	14.3	12.9	12.4	13.0	**0.0**							
11) Pgig	13.5	12.8	12.7	13.1	13.1	13.4	13.9	13.8	12.9	10.2	**0.0**						
12) Hlep	12.9	11.9	12.0	11.9	12.0	13.2	12.9	13.0	11.9	13.8	14.4	**0.0**					
13) Htyp	12.4	11.9	11.9	11.9	12.2	13.2	12.7	12.9	12.1	13.4	14.3	1.4	**-**				
14) Hwaa	12.6	11.8	11.9	11.9	12.1	12.9	12.8	12.9	11.9	13.6	14.1	0.5	1.4	**-**			
15) Llon	16.2	17.5	17.0	17.2	17.7	16.9	17.0	17.6	18.1	16.9	17.9	17.4	16.8	17.4	**0.0**		
16) Lhex	16.9	18.0	17.9	17.6	18.3	17.5	17.8	18.7	18.2	17.8	18.3	17.1	16.7	16.9	2.7	**-**	
17) Cpra	16.6	17.6	17.6	16.6	17.3	16.6	17.0	17.4	17.9	17.4	17.2	16.6	17.1	16.4	11.7	12.9	**0.0**

Table 11 (Continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
18) Csin	15.9	17.1	17.9	15.9	16.7	16.4	16.9	17.1	17.4	17.0	16.4	17.6	17.6	17.5	9.0	9.4	10.5	**0.4**				
19) Esal	17.4	18.0	17.9	16.6	17.1	16.6	17.0	18.3	16.2	17.9	18.2	17.2	17.7	17.2	14.0	14.6	11.3	12.9	**0.2**			
20) Pmoo	18.5	18.0	17.8	18.4	17.6	17.5	17.9	18.5	17.3	18.5	18.5	18.4	18.8	18.6	16.5	17.1	15.5	17.1	16.7	-		
21) Pbra	18.5	17.7	18.5	19.2	19.1	18.4	19.8	19.4	18.1	19.0	18.8	18.6	18.5	18.5	16.8	16.7	17.7	16.6	17.5	14.0	-	
22) Ipun	18.0	17.9	17.6	18.4	18.1	18.4	18.3	18.6	17.8	17.3	17.3	18.6	18.2	18.5	19.5	19.8	20.1	18.8	20.4	19.8	19.4	-

Annotation: Pboc = *Pangasius bocourti*, Pmac = *P. macronema*, Ppol = *P. polyuranodon*, Pcon = *P. conchophilus*, Pkre = *P. krempfi*, Plan = *P. larnaudii*, Psan = *P. sanitwongsei*, Pnas = *P. nasutus*, Pple = *Pseudolais pleurotaenia*, Phyp = *Pangasianodon hypophthalmus*, Pgig = *P. gigas*, Hlep = *Helicophagus leptorhynchus*, Htyp = *H. typus*, Hwaa = *H. waandersii*, Llon = *Laides longibarbis*, Lhex = *L. hexanema*, Cpra = *Clupisoma prateri*, Csin = *C. sinense*, Esal = *Eutropiichthys salweenensis*, Pmoo = *Pseudeutropius moolenburghae*, Pbra = *P. brachypopterus*, Ipun = *Ictalurus punctatus.* Hyphen (-) indicates the value did not obtain, since only one sequence for each species was included for the analysis.

Table 12 The values of *cyt b p*-distance (in percentage) between genera of pangasiids and schilbeids

	1	2	3	4	5	6	7	8	9
1. *Pangasius*									
2. *Pseudolais*	11.4								
3. *Pangasianodon*	13.2	13.0							
4. *Helicophagus*	12.4	12.0	14.0						
5. *Laides*	17.4	18.2	17.6	17.1					
6. *Clupisoma*	16.9	17.6	17.0	17.1	10.6				
7. *Eutropiichthys*	17.4	16.2	18.1	17.3	14.2	12.1			
8. *Pseudeutropius*	18.4	17.7	18.7	18.5	16.7	16.7	17.1		
9. *Ictalurus*	18.2	17.8	17.3	18.4	19.6	19.5	20.4	19.6	

B. RNA data set

The mt ribosomal genes evolve at a slower rate than mt protein coding genes (Meyer 1993). Thus, the *p*-distance value which is estimated from *12S* and/or *16S rRNA* genes is much lower than that observed for the *cyt b* gene as seen in the previous studies (Ortí *et al*., 1996; Ritchie *et al*., 1996; Šlechtová *et al*., 2006). At the intergeneric leval, *p*-distances among genera of the subfamily Serrasalminae (piranhas), estimating from combined *12S* and *16S rRNA* sequences ranged from 0.9% to 8.9% (Ortí *et al*., 1996). At the interspecific level, *p*-distances among species of the same genus of the subfamily Serrasalminae ranged from 0.1% to 5.8% (Ortí *et al*., 1996). The corresponding values were also found in the Antarctic fish genus *Trematomus* (0.5%-3.9%; Ritchie *et al*., 1996), the botiid loaches genus *Yasuhikotakia* (mean = 4.17%; Šlechtová *et al*., 2006) and the gobiesocid fishes (Gobiesocidae, mean = 4.0%; Almada *et al*., 2008). At the intraspecific level, the variation within botiid species (Botiidae) ranged from 0.0% to 0.75% (Šlechtová *et al*., 2006).

In the present study, the sequence of *12S rRNA*-tRNA^{Val}-*16S rRNA* of 35 pangasiid and schilbeid specimens from this study along with one sequence of

outgroup species, *Ictalurus punctatus* (AF482987; Waldbieser, 2003) were used for *p*-distance estimation. The resulting pairwise *p*-distances were averaged and were presented in the matrix of the percentage interspecific and intergeneric *p*-distances of pangasiids and schilbeids.

At the interfamilial level, the *p*-distance values between pangasiids and schilbeids ranged from 7.4% to 9.5% (Table 13) with an average of 8.4%. Within the family Pangasiidae, the intergeneric *p*-distance ranged from 4.0% between *Helicophagus* and *Pseudolais* to 5.4% between *Pangasianodon* and *Helicophagus* (Table 14), with a mean of 4.7%. These values were higher than those reported between genera within hagfishes (Myxinidae, 2.25%; Kuo *et al*., 2003). However, the intergeneric *p*-distance among Pangasiidae in this study fell within the range of *p*-distance among genera of the same species reported for the subfamily Serrasalminae (0.9%-8.9%; Ortí *et al*., 1996) and approached to the mean sequence divergence among genera of Botiidae (5.85%; Šlechtová *et al*., 2006). This result indicates the generic status of *Pangasius*, *Helicophagus*, *Pseudolias* and *Pangasianodon*.

At the interspecific level, the *p*-distance values between species for each pangasiid genus were reported as: *Pangasius*, 1.9 – 4.7%; *Pangasianodon*, 3.7% and *Helicophagus*, 0.2% (Table 13). This is except for the genus *Pseudolais* in which only one species was included in this analysis. The values of interspecific sequence divergenece among Pangasiidae reported in this study are consistent with the sequence divergences between species of the same genus as previously reported in Antarctic fish genus *Trematomus* (0.5% - 3.9%; Ritchie *et al*., 1996), botiid loaches (Botiidae) (mean = 4.17% for the genus *Yasuhikotakia* and 4.91% for *Syncrossus*; Šlechtová *et al*., 2006), lutjanid fishes (Lutjanidae) (mean = 0.2% for *Pterocaesio* and 0.36% for *Lutjanus*; Miller and Cribb, 2007) and gobiesocid fishes (Gobiesocidae) (mean = 4.0%; Almada *et al*., 2008). This result confirms the species status of pangasiid taxa used in this study.

Regarding the intraspecific variation within Pangasiidae, there was no genetic difference between two individuals of eight species including *Pangasius*

polyuranodon, *P. conchophilus*, *P. krempfi*, *P. sanitwongsei*, *Pseudolais pleurotaenia*, *Pangasianodon hypophthalmus*, *P. gigas*, *Helicophagus leptorhynchus* (Table 13), whereas the intraspecific variation with 0.1% was found in three species including *Pangasius bocourti*, *P. macronema*, *P. larnaudii*. These intraspecific variations were low and generally fell within reported ranges of within botiid species *rRNA* divergence (0.0%-0.75%; Šlechtová *et al*., 2006). This result confirms the validity of specimens for each recognized pangasiid species used in this study.

Within the family Schilbeidae, the intergeneric *p*-distances ranged from 3.3% (*Laides* and *Clupisoma*) to 9.5% (*Eutropiichthys* and *Pseudeutropius*) (Table 14), with a mean of 6.8%. These values were higher than those reported between genera of Pangasiidae (mean = 4.7%) from this study and of Myxinidae (mean = 2.25%; Kuo *et al*., 2003). However, the intergeneric *p*-distance among Schilbeidae in this study fell within the range of *p*-distance among genera of the subfamily Serrasalminae (0.9%-8.9%; Ortí *et al*., 1996). This result indicates the generic status of *Laides*, *Clupisoma*, *Eutropiichthys* and *Pseudeutropius.*

At the interspecific level, *p*-distances among species within each schilbeid genus were determined as follows: *Clupisoma*, 3.7% and *Pseudeutropius*, 6.8% (Table 13). This is except for the genus *Laides* and *Eutropiichthys* in which only one species was included in this analysis. Athough the interspecific sequence divergenec among *Pseudeutropius* (6.8%) was higher than those reported in Pangasiidae (0.2%-4.7%), however, this value fell within the range of *p*-distance among species of the genus Myxine (0.7%-8.1%) of the family Myxinidae (Kuo *et al*., 2003). As with found in *cyt b p*-distance, *Clupisoma sinense* was closely related with *Laides longbarbis* with 2.8% sequence divergence rather than its congener, *C. preteri* with 3.7% sequence divergence. This result indicates that *C. sinense* and *L. longibarbis* most likely belong to the same genus.

To validate the putative new schilbeid species status of *Eutropiichthys salweenensis* which was formerly recognized as *E. vacha* (Vidthayanon and Roongthongbaisuree, 1993), the available partial sequence (448 bp) of *16S rRNA* gene

of *E. vacha* was retrived from GenBank (GQ357917) and was used for estimating the average sequence divergence between *E. vacha* and *E. salweenensis* from this study. Because the *16S rRNA* sequence of *E. vacha* is relatively short (465 bp) compared with the RNA data set in this study (approximately 1,850 bp of *12S rRNA*-tRNA^{Val}-*16S rRNA*), thus the RNA sequence of *E. salweenensis* from this study was trimmed to the size of the smallest fragment (465 bp) to maintain consistency of the data. The mean *p*-distance between these two species was 2.2%. This value fell within the range of the interspecific *p*-distance within Pangasiidae (0.2%-4.7%) reported in this study, the Antarctic fish genus *Trematomus* (0.5% - 3.9%; Ritchie *et al*., 1996) and corresponded to the mean interspecific sequence divergence of the genus *Sinibotia* (2.03%; Šlechtová *et al*., 2006). This result suggests that *E. salweenensis* is genetically distinct from *E. vacha* and the species status of the former as previously suggested by Ferraris and Vari (2007) is confirmed in this study. Based on morphological data according to Ferraris and Vari (2007), *E. salweenensis* differed from *E. vacha* in the combination of the number of branched anal fin rays (48-50 vs. 44-48), the form of the lateral margin of the pectoral spine (smooth in *E. salweenensis* vs. roughened in *E. vacha*) and the form of the snout in lateral view (rounded in *E. salweenensis* vs. pointed in *E. vacha*). *E. salweenensis* is only found in Salween River in Thailand, whereas *E. vacha* distributes in the region from eastern Pakistan to Calcutta, India.

Regarding the intraspecific variation within Schilbeidae, there was no genetic difference between specimens in two species including *Laides longibarbis* and *Clupisoma prateri* (Table 13), whereas the intraspecific variation was observed in *Eutropiichthys salweenensis* (0.1%) and *Clupisoma sinense* (0.2%). These intraspecific variations were low and generally fell within reported ranges of within botiid species *rRNA* divergence (0.0%-0.75%; Šlechtová *et al*., 2006). The small values of sequence divergence among individuals within each schilbeid species suggested that the specimens for each recognized species used in this study belong to the same species.

Table 13 The values of RNA *p*-distance (in percentage) between species of pangasiids and schilbeids (below diagonal) and within each species (diagonal values in bold font)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1) Pboc	**0.1**																
2) Pmac	4.0	**0.1**															
3) Ppol	4.2	2.2	**0.0**														
4) Pcon	4.1	4.0	4.6	**0.0**													
5) Pkre	3.7	4.4	4.4	3.5	**0.0**												
6) Plar	3.7	4.2	4.7	3.9	3.2	**0.1**											
7) Psan	3.3	3.7	3.9	3.5	3.2	3.1	**0.0**										
8) Pnas	3.9	4.1	4.6	1.9	3.4	3.6	3.3	-									
9) Pple	4.4	4.6	5.0	4.5	3.8	4.3	3.7	4.3	**0.0**								
10) Phyp	4.7	4.9	4.9	4.6	4.6	5.1	4.6	4.7	5.3	**0.0**							
11) Pgig	5.0	5.1	5.1	4.5	4.7	5.1	4.6	4.8	4.7	3.7	**0.0**						
12) Hlep	4.3	4.9	4.9	4.7	4.0	4.3	4.2	4.2	3.9	5.3	5.4	**0.0**					
13) Htyp	4.5	5.1	5.1	4.8	4.0	4.4	4.3	4.3	4.0	5.4	5.5	0.2	-				
14) Llon	8.9	8.6	8.5	8.6	8.1	8.2	8.4	8.7	8.7	8.6	8.0	8.9	8.9	**0.0**			
15) Cpra	8.8	8.8	8.5	8.5	7.7	8.4	8.5	8.4	8.7	8.6	8.3	8.7	8.8	3.8	**0.0**		
16) Csin	8.9	8.8	8.6	8.7	8.2	8.4	8.5	8.7	8.8	8.9	8.5	9.1	9.1	2.8	3.7	**0.2**	
17) Esal	9.3	9.3	8.9	9.2	8.7	9.4	9.5	9.2	9.1	8.9	8.9	9.2	9.3	4.8	4.0	5.1	**0.1**

Table 13 (Continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
18) Pmoo	7.9	8.2	8.3	8.3	7.5	7.9	8.0	8.2	8.2	7.4	8.1	7.9	7.8	9.5	9.7	9.7	9.8	-		
19) Pbra	8.4	8.2	8.5	8.4	7.8	8.1	8.1	8.5	8.0	7.7	8.3	8.1	8.2	9.1	9.1	8.9	9.3	6.8	-	
20) Ipun	8.0	8.5	8.3	7.8	7.7	7.9	7.9	7.8	8.0	7.4	7.2	8.0	8.1	9.6	9.6	9.8	10.7	9.6	9.1	-

Annotation: Pboc = *Pangasius bocourti*, Pmac = *P. macronema*, Ppol = *P. polyuranodon*, Pcon = *P. conchophilus*, Pkre = *P. krempfi*, Plan = *P. larnaudii*, Psan = *P. sanitwongsei*, Pnas = *P. nasutus*, Pple = *Pseudolais pleurotaenia*, Phyp = *Pangasianodon hypophthalmus*, Pgig = *P. gigas*, Hlep = *Helicophagus leptorhynchus*, Htyp = *H. typus*, Hwaa = *H. waandersii*, Llon = *Laides longibarbis*, Lhex = *L. hexanema*, Cpra = *Clupisoma prateri*, Csin = *C. sinense*, Esal = *Eutropiichthys salweenensis*, Pmoo = *Pseudeutropius moolenburghae*, Pbra = *P. brachypopterus*, Ipun = *Ictalurus punctatus.* Hyphen (-) indicates the value did not obtain, since only one sequence for each species was included for the analysis.

Table 14 The values of RNA *p*-distance (in percentage) between genera of pangasiids and schilbeids

	1	2	3	4	5	6	7	8	9
1. *Pangasius*									
2. *Pseudolais*	4.3								
3. *Pangasianodon*	4.8	5.0							
4. *Helicophagus*	4.5	4.0	5.4						
5. *Laides*	8.5	8.7	8.3	8.9					
6. *Clupisoma*	8.5	8.7	8.6	8.9	3.3				
7. *Eutropiichthys*	9.2	9.1	8.9	9.2	4.8	4.5			
8. *Pseudeutropius*	8.1	8.1	7.9	8.0	9.3	9.3	9.5		
9. *Ictalurus*	8.0	8.0	7.3	8.0	9.6	9.7	10.7	9.4	

C. *RAG1* gene

Substitution rate of the nuclear *RAG1* gene was relatively slower than that of almost all mt protein genes (Groth and Barrowclough, 1999; Rüber *et al*., 2004; San Mauro *et al*., 2004). Thus, the sequence divergence (*p*-distance) values estimating from the nuclear *RAG1* sequence were much lower than those observed in the *cyt b* and the RNA data.

Based on *RAG1* gene sequences, all of 35 sequences of pangasiid and schilbeid specimens along with one sequence of outgroup species, *Ictalurus punctatus* (AF482987; Waldbieser, 2003) were used for *p*-distance estimation. The resulting pairwise *p*-distances were averaged and were presented in the matrix of the percentage interspecific and intergeneric *p*-distances of pangasiids and schilbeids.

At the interfamilial level, the *p*-distance values between pangasiids and schilbeids ranged from 5.6%-7.9% (Table 15), with an average of 7.0%. Within the family Pangasiidae, the intergeneric *p*-distances ranged from 2.0% (*Pangasius* and *Pseudolais*) to 2.7% (*Pangasius* and *Pangasianodon*) (Table 16) with a mean of 2.3%. These values approached with the mean sequence divergence between cichlid

fish genera *Caquetaia* and *Theraps* (mean = 1.7%; Hulsey *et al*., 2010). As with *cyt b* and RNA sequence divergences, this result confirms the generic status of *Pangasius Helicophagus*, *Pseudolais* and *Pangasianodon*.

Regarding the interspecific variation within Pangasiidae, the *p*-distance values between species for each genus were reported as: *Pangasius*, 0.1 – 1.9%; *Pangasianodon*, 1.5% and *Helicophagus*, 0.2% (Table 15). This is except for the genus *Pseudolais* in which only one species was included in this analysis. The interspecific variation among Pangasiidae fell within the range of the sequence divergences between species of the same genus in cyprinid fishes (0.1-1.3% within *Tampichthys*, 0.06-2.2% within *Cyprinella*, 1.6% within *Hybognathus* and 0.06-1.3% within *Dionda*; Schönhuth *et al*., 2008). This result confirms the species status of pangasiid taxa used in this study. The intraspecific variation was observed in three out of all 13 pangasiid species including *Pangasius bocourti* (0.1%), *P. macronema* (0.1%) and *Helicophagus leptorhynchus* (0.1%). There is no genetic difference between two individuals in the remaining species, except for *H. typus* in which only one specimen was included for estimating sequence divergence.

Within the family Schilbeidae, the intergeneric *p*-distance ranged from 2.1% (*Laides* and *Clupisoma*) to 8.0% (*Eutropiichthys* and *Pseudeutropius*) (Table 16), with a mean of 5.0%. Although the sequence divergence between *Eutropiichthys* and *Pseudeutropius* (8.0%) was much higher than those found in Pangasiidae (mean = 2.3%), however, this value corresponded to the maximum value (12.8%) of *RAG1* sequence divergence between the genera of reef fish family Pomacentridae (Cooper *et al*., 2009). Thus, this result confirms the generic status of *Laides*, *Clupisoma*, *Eutropiichthys* and *Pseudeutropius*.

At the interspecific level, the sequence divergences among species within each schilbeid genus were: *Clupisoma*, 1.9% and *Pseudeutropius*, 2.4% (Table 15). This is except for the genera *Laides* and *Eutropiichthys* in which only one species was included in this analysis. The interspecific variation among Schilbeidae corresponded or approached to the interspecific sequence divergences in cyprinid fishes (0.06-2.2%

within *Cyprinella* and 1.6% within *Hybognathus*; Schönhuth *et al*., 2008). Like in *cyt b* and RNA data set, the result of nuclear *RAG1* sequence divergence also indicates the probability that *Clupisoma sinense* and *Laides longibarbis* should belong to the same genus. This is illustrated by the small sequence divergence between *C. sinense* and *L. longibarbis* (1.7%) which is less than that between *C. sinense* and its congener, *C. preteri* (1.9%). Regarding the intraspecific variation within the family Schilbeidae, there is no genetic change between two individuals of *Clupisoma prateri*, whereas the intraspecific variation was observed in *L. longibarbis* (0.1%), *C. sinense* (0.1%) and *E. salweenensis* (0.1%).

Table 15 The values of *RAG1 p*-distance (in percentage) between species of pangasiids and schilbeids (below diagonal) and within each species (diagonal values in bold font)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1) Pboc	**0.1**																
2) Pmac	1.9	**0.1**															
3) Ppol	1.6	0.6	**0.0**														
4) Pcon	1.0	1.2	0.8	**0.0**													
5) Pkre	1.6	1.7	1.4	0.8	**0.0**												
6) Plar	1.1	1.5	1.2	0.6	1.2	**0.0**											
7) Psan	0.9	1.0	0.7	0.1	0.7	0.5	**0.0**										
8) Pnas	1.1	1.3	0.9	0.3	0.9	0.7	0.2	**0.0**									
9) Pple	2.3	2.4	2.1	1.5	2.1	1.9	1.4	1.6	**0.0**								
10) Phyp	2.6	3.0	2.7	2.1	2.2	2.2	2.0	2.2	2.2	**0.0**							
11) Pgig	3.4	3.5	3.1	2.7	3.0	3.0	2.6	2.8	2.8	1.5	**0.0**						
12) Hlep	2.4	2.7	2.3	1.9	2.3	2.0	1.8	1.8	2.0	2.2	3.0	**0.1**					
13) Htyp	2.4	2.7	2.3	1.9	2.2	2.0	1.7	1.7	2.0	2.1	2.9	0.2	-				
14) Llon	7.5	7.8	7.5	6.9	7.5	7.2	7.1	6.9	7.2	6.2	7.2	7.1	7.2	**0.1**			
15) Cpra	6.7	7.2	6.9	6.1	6.6	6.3	6.2	6.3	6.5	5.6	6.5	6.6	6.5	2.3	**0.0**		
16) Csin	7.5	7.8	7.5	6.6	7.2	6.9	6.8	6.9	6.8	5.9	6.9	6.9	6.9	1.7	1.9	**0.1**	
17) Esal	7.7	7.9	7.6	6.8	7.2	7.1	6.9	7.1	7.3	5.9	7.1	7.1	7.1	2.9	1.8	2.8	**0.1**

Table 15 (Continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
18) Pmoo	7.3	7.2	7.1	6.6	7.2	6.9	6.8	6.8	7.3	7.1	7.3	6.9	6.9	7.5	6.6	7.1	7.8	-		
19) Pbra	7.4	7.2	7.1	7.0	7.3	7.0	6.9	6.9	7.3	7.1	7.6	7.1	7.1	7.3	7.3	7.7	8.2	2.4	-	
20) Ipun	5.7	6.1	5.5	5.4	5.7	5.4	5.2	5.2	5.5	5.2	5.8	5.1	5.1	7.7	7.0	7.5	8.0	7.5	7.5	-

Annotation: Pboc = *Pangasius bocourti*, Pmac = *P. macronema*, Ppol = *P. polyuranodon*, Pcon = *P. conchophilus*, Pkre = *P. krempfi*, Plar = *P. larnaudii*, Psan = *P. sanitwongsei*, Pnas = *P. nasutus*, Pple = *Pseudolais pleurotaenia*, Phyp = *Pangasianodon hypophthalmus*, Pgig = *P. gigas*, Hlep = *Helicophagus leptorhynchus*, Htyp = *H. typus*, Hwaa = *H. waandersii*, Llon = *Laides longibarbis*, Lhex = *L. hexanema*, Cpra = *Clupisoma prateri*, Csin = *C. sinense*, Esal = *Eutropiichthys salweenensis*, Pmoo = *Pseudeutropius moolenburghae*, Pbra = *P. brachypopterus*, Ipun = *Ictalurus punctatus.* Hyphen (-) indicates the value did not obtain, since only one sequence for each species was included for the analysis.

Table 16 The values of *RAG1 p*-distance (in percentage) between genera of pangasiids and schilbeids.

	1	2	3	4	5	6	7	8	9
1. *Pangasius*									
2. *Pseudolais*	2.0								
3. *Pangasianodon*	2.7	2.5							
4. *Helicophagus*	2.1	2.0	2.5						
5. *Laides*	7.3	7.2	6.7	7.1					
6. *Clupisoma*	6.8	6.7	6.2	6.7	2.1				
7. *Eutropiichthys*	7.3	7.3	6.5	7.1	2.9	2.3			
8. *Pseudeutropius*	7.0	7.3	7.3	7.0	7.4	7.2	8.0		
9. *Ictalurus*	5.5	5.5	5.5	5.1	7.7	7.3	8.0	7.5	

8. Phylogenetic analysis

In the present study, phylogenetic relationships of pangasiids and schilbeids based on *cyt b*, RNA, *RAG1* and combined data were reconstructed with different analytic methods (NJ, MP, ML and BI).

A. *Cyt b* gene

The *cyt b* sequences of 38 taxa including 35 pangasiid and schilbeid specimens in this study and three *cyt b* sequences retrieved from GenBank (*Helicophagus waandersii*; DQ119468, *Laides hexanema*; EU490915 and outgroup: *Ictalurus punctatus*; AF482987) were aligned. The *cyt b* data set consisted of 1,106 sites were used for phylogenetic analyses. The *cyt b* phylogenetic trees obtained from NJ, MP, ML and BI methods as illustrated in Figure 12-15 are slightly different. NJ and ML analyses yielded the similar topological trees as shown in Figure 12 and Figure 13, respectively. In NJ and ML trees, two main monophyltic clades corresponding to the families Pangasiidae and Schilbeidae were recognized with a highly statistical confidence (93%-100%). Within pangasiid clade, four subclades corresponding to the genera *Pangasius*, *Pseudolais*, *Helicophagus* and

Pangasianodon were recovered. *Pangasius* appeared to be the monophyletic lineage but with low statistical confidence (64% and <50% in NJ and ML trees, respectively). It was the most recent diverged group of pangasiids. The branching orders among *Pangasius* species was largely unresolved, however, the close relatedness between *Pangasius macronema* and *P. polyuranodon* and between *P. conchophilus* and *P. nasutus* was highly supported. The *Pseudolais* seemed to be the sister lineage of *Pangasius* in NJ analysis (<50% of bootstrap support), but changed to aggregate with *Helicophagus* in ML tree with the higher statistical support (54%). *Helicophagus* was strongly supported as monophyletic assemblage. *H. leptorhynchus* grouped well with *H. waandersii* and then *H. typus* appeared to be the basal taxon of *Helicophagus*. Also, *Pangasianodon* was highly supported as monophyletic group as the basal lineage of the family Pangasidae with a robustly statistical confidence (97% and 93% in NJ and ML trees, respectively). Within schilbeid clade, *Clupisoma* was recognized as polyphyletic assemblage. *C. sinense* closely affiliated to *Laides* with highly statistical support (94% in NJ and 97% in ML), whereas *C. prateri* aggregated with *Eutropiichthys salweenensis* but with low statistical confidence (63% and 60% in NJ and ML, respectively). *L. longibarbis* was highly supported as sister to *L. hexanema. Pseudeutropius* was placed as the basal of Schilbeidae.

MP and BI trees as shown in Figure 14 and Figure 15 are seemingly identical. Two major monophyletic clades, Pangasiidae and Schilbeidae, were still highly supported (90%-100%). Within pangasiid clade, the topologies appeared in MP and BI trees different from those found in NJ and ML analyses. *Pangasius* was recovered as polyphyletic. *Helicophagus* and *Pseudolais* were embedded within the same clade with *Pangasius*. Their branching orders were unresolved because the polytomy branches have been occurred among them. *Heligophagus* and *Pseudolais* had a tendency to cluster in BI tree but were separated from each other in MP. In MP and BI trees, there was strong support for monophyly of *Helicophagus* like in NJ and ML analyses. *H. leptorhynchus* was still highly supported as sister to *H. waandersii* (100%). *H. typus* was still as the sister lineage in this group. In all analyses, *Pangasianodon* was monophyletic lineage and was the basal group of the family Pangasiidae. Within schilbeid clade, MP and BI analyses contributed the same

topologies with NJ and ML trees. *Pseudeutropius* was still at the base of the clade. *Clupisoma sinense* aggregated well with *L. longibarbis* and *L. hexanema*. *C. prateri* was associated with *E. salweenensis* in MP tree but unresolved in BI analysis.

As mentioned above, all phylogenetic analyses (NJ, MP, ML and BI) inferred from *cyt b* sequences contributed the high resolution for monophyletic assemblages of Pangasiidae and Schilbeidae (90%-100% statistical support values at 8 interior branches from NJ, MP, ML and BI trees, Table 17), indicating that *cyt b* is the effective marker for resolving taxonomic classification at the family level (Meyer, 1994; Peng *et al*., 2004; Doiuchi and Nakabo, 2006; Perdices *et al*., 2008). Within pangasiid clade, *Pangasianodon* clearly separated from the other pangasiids and was recovered as the basal lineage. This result is inconsistent with previous molecular phylogenies based on allozyme and *cyt b* (Pouyaud *et al*., 2000) and *12S rRNA* data (Pouyaud *et al*., 2004). Pouyaud *et al*. (2000) proposed *Pteropangasius* (or *Pseudolais*) and *Helicophagus* as the basal group and *Pangasianodon* as more derived lineage. Pouyaud *et al*. (2004) indicated that *Pteropangasius* (or *Pseudolais*) as the most basal lineage of pangasiids and *Pangasianodon* as sister group to *Pangasius* + *Helicophagus*. The interrelationships among *Pangasius*, *Pseudolais* and *Helicophagus* in *cyt b* phylogenies were less resolved in the present study, indicating that *cyt b* sequences might have less variation to resolve intergeneric relationships among pangasiids. Therefore, increasing the number of nucleotide characters from the other genetic loci should provide more resolved and robust phylogeny. Within *Pangasius*, the relationships among species were mostly unresolved in all analyses, except in the case of the sister group relationships between *P. macronema* + *P. polyuranodon* and *P. conchophilus* + *P. nasutus*. Several studies (Pollock *et al*., 2002; Zwickl and Hillis, 2002; Heath *et al*., 2008) have indicated that introducing additional taxa into a phylogenetic analysis will increase the accuracy of the inferred topology by dispersing homoplasy across the tree and reducing the effect of long-branch attraction. Moreover, additional sampling taxa also improves parameter estimation for evolutionary model which will provide better descriptions of evolutionary history of the organisms and is thus important for improved applications of model-based methods (NJ, ML and BI). Lack of resolution within *Pangasius* may be likely to

incomplete taxon sampling (Geuten *et al*., 2004). Therefore, a broader representation of the *Pangasius* species which distribute beyond the borders of Thailand in total of 15 species should give better resolution.

Within *Helicophagus*, *H. leptorhynchus* and *H. waandersii* have been defined as two independent species by Ng and Kottelat (2000). In this study, the new putative species "*H. leptorhynchus*" was grouped to *H. waandersii* in all trees. They were genetically different with 0.5% of *cyt b* sequence divergence. This value corresponded to the intraspecific sequence divergence found in goodeid fishes (0.01%-1.7%; Doadrio and Domínguez, 2004) as well as in the cuban fishes genus *Girardinus* (0.09%-1.1%; Doadrio *et al*., 2009). This result suggests that *H. leptorhynchus* (Ng and Kottelat, 2000) might consider as the synonym of *H. waandersii* Bleeker (1858).

Within schilbeid clade, all phylogenetic analyses contributed the stable topology with the *Pseudeutropius* as basal taxon of the clade. *C. sinense* was highly grouped with *Laides* clade rather than *C. prateri*, supporting that this species should belong to the genus *Laides* as suggested by Kottelat (1989), Zakaria-Ismail (1992) and Rainboth (1996). Within *Laides*, *L. longibarbis* and *L. hexanema* have been defined as two distinct species by Ng (1999). This study also confirms the new species status of *L. longibarbis*. It was sister taxon of *L. hexanema* in all analyses. These two species were genetically different with 2.7% of *cyt b* sequence divergence. This value corresponded to the interspecific *p*-distances found in goodeid fishes (1.7%-11%; Doadrio and Domínguez, 2004) and in southern North American cyprinids (2.1%-11.4%; Schönhuth *et al*., 2008).

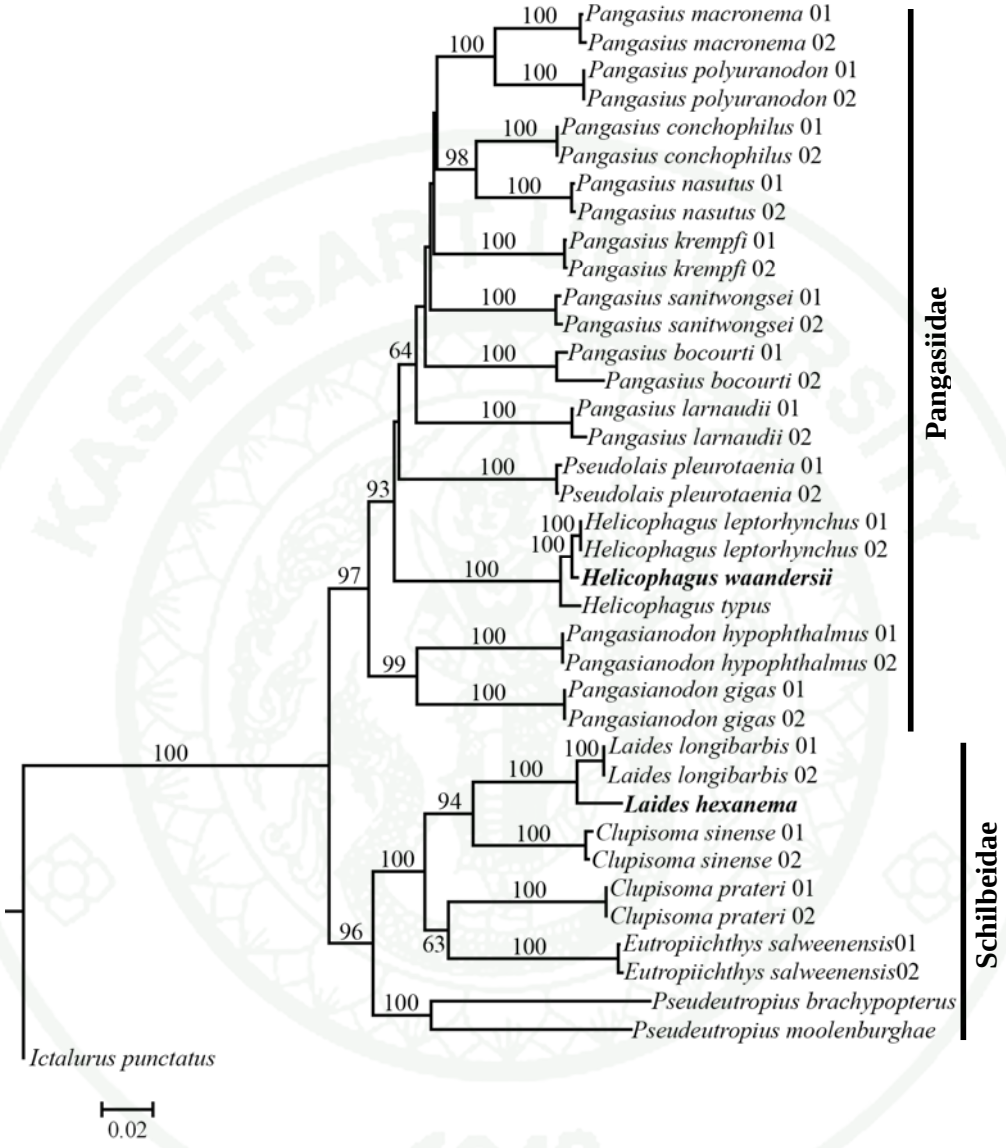


Figure 12 NJ tree of Thai pangasiids and schilbeids inferred from 1,106 bp of *cyt b* sequences. Names in regular font represent samples used in this study. The sequences from the previous studies (*Helicophagus waandersii*; DQ119468 and *Laides hexanema*; EU490915) were included and the sample names represent in bold font. The scale bar represents 0.02 substitution/site. Numbers above branches represent bootstrap support for NJ (1000 replicates). Nodes with support value below 50% are not numbered.

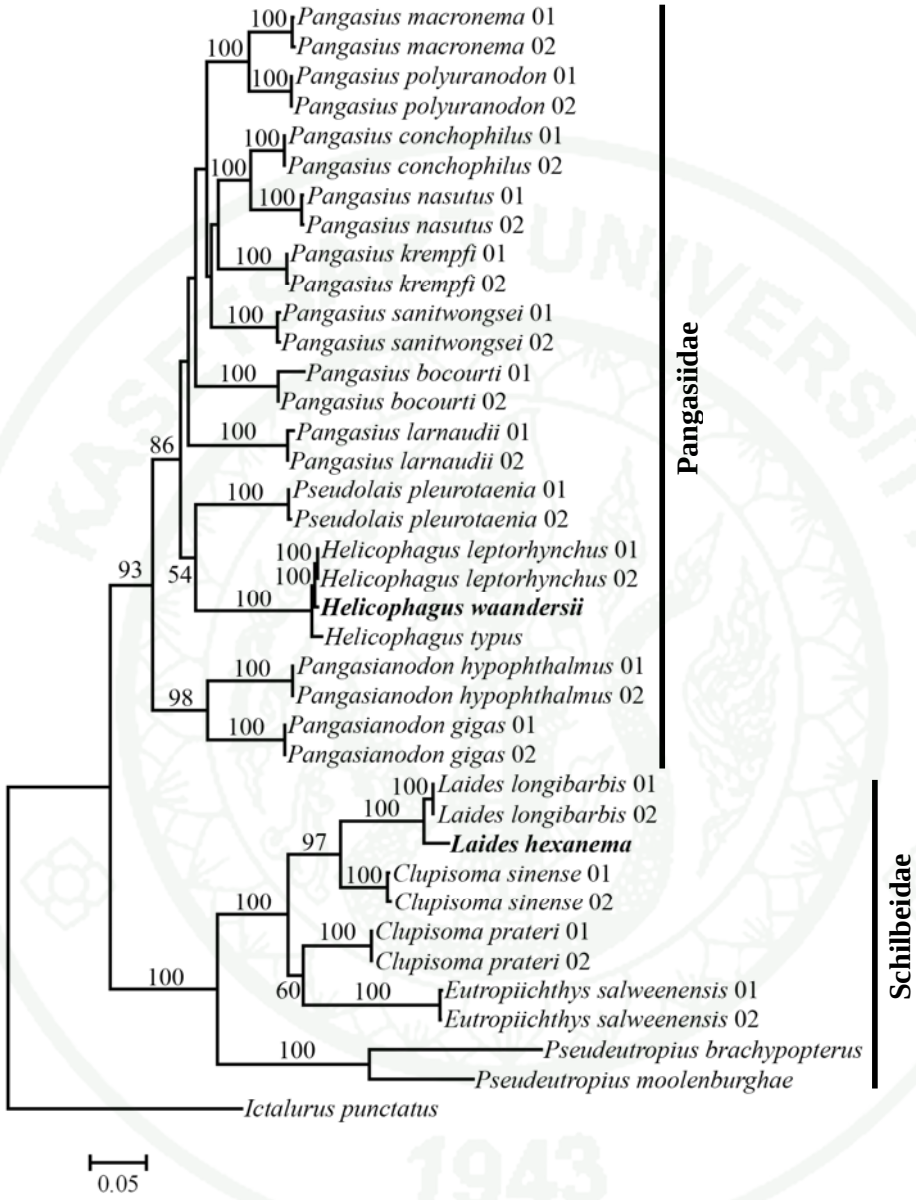


Figure 13 ML tree of Thai pangasiids and schilbeids inferred from 1,106 bp of *cyt b* sequences. Names in regular font represent samples used in this study. The sequences from the previous studies (*Helicophagus waandersii*; DQ119468 and *Laides hexanema*; EU490915) were included and the sample names represent in bold font. The scale bar represents 0.05 substitution /site. Numbers above branches represent bootstrap support for ML (500 replicates). Nodes with support value below 50% are not numbered.

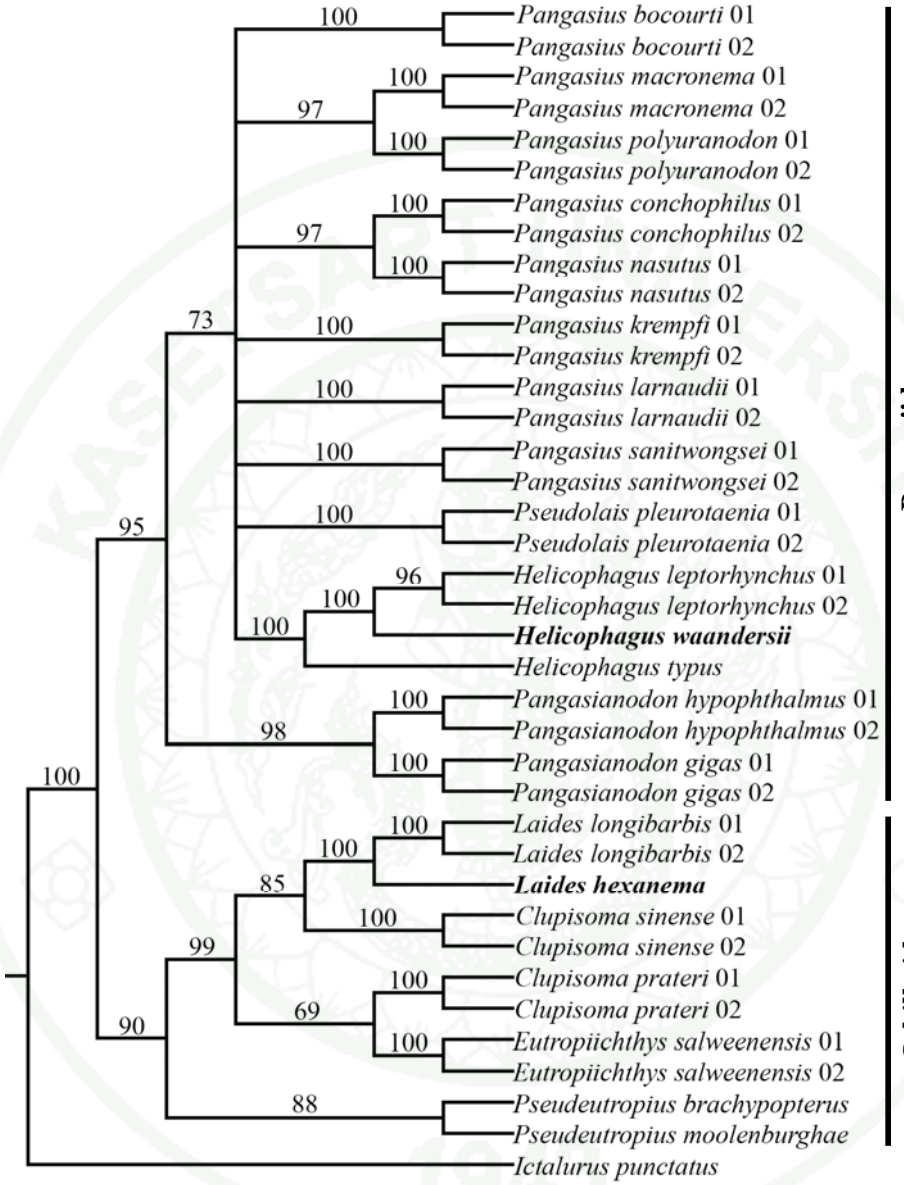


Figure 14 Consensus MP tree of Thai pangasiids and schilbeids inferred from 1,106 bp of *cyt b* sequences. Names in regular font represent samples used in this study. The sequences from the previous studies (*Helicophagus waandersii*; DQ119468 and *Laides hexanema*; EU490915) were included and the sample names represent in bold font. Numbers above branches represent bootstrap support (1000 replicates) for MP.

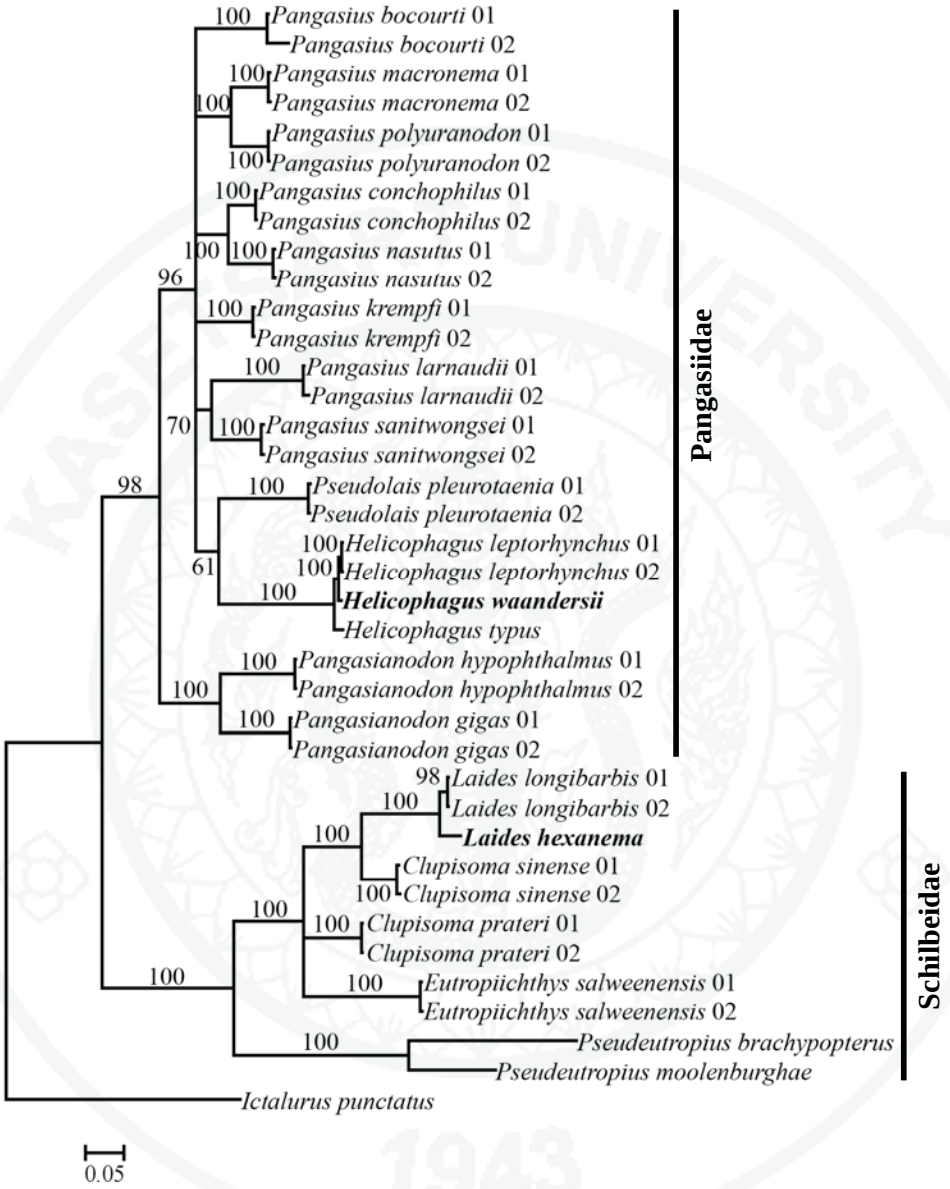


Figure 15 BI tree of Thai pangasiids and schilbeids inferred from 1,106 bp of *cyt b* gene. Names in regular font represent samples used in this study. The sequences from the previous studies (*Helicophagus waandersii*; DQ119468 and *Laides hexanema*; EU490915) were included and the sample names represent in bold font. The scale bar represents 0.05 substitution/site. Numbers above branches represent the posterior probability percentage for BI.

B. RNA data set

The RNA sequences of 35 taxa of pangasiids and schilbeids from this study and one outgroup species, *Ictalurus punctatus* (AF482987) were aligned. The RNA data set composed of 1,850 nucleotide characters were used for phylogenetic analyses with NJ, MP, ML and BI methods.

The RNA phylogenies obtained from NJ, MP, ML and BI methods as illustrated in Figure 16-19 are somewhat different in detail. NJ topology based on RNA data set (Figure 16) supports the recognition of two main monophyletic clades corresponding to Pangasiidae and Schilbeidae. There are two major groups in pangasiid clade; *Pangasianodon* was strongly supported (83%) as basal taxon in this clade. The other one consists of *Pangasius*, *Helicophagus* and *Pseudolais*. *Pangasius* appeared polyphyletic assemblage. The *Heligophagus* and *Pseudolais* had a trend to cluster in NJ tree with poorly statistical support (<50%) and they nested within the *Pangasius*. Within schilbeid clade, NJ analysis highly supported (99%) the close affiliation of *Laides longibarbis* and *Clupisoma sinense* with *C. prateri* as a sister taxon. *Eutropiichthys salweenensis* was recognized as sister group containing *L. longibarbis*, *C. sinense* and *C. prateri* with 100% of bootstrap support value. *Pseudeutropius* was the basal lineage of the Schilbeidae.

MP topology inferred from the RNA data set is shown in Figure 17. It demonstrates also two major clades, Pangasiidae and Schilbeidae with 77% and 78% of statistical support values, respectively. As found in NJ tree, *Pangasius* was polyphyletic assemblage. *Helicophagus* was monophyletic and separated from *Pseudolais* but it still placed in the *Pangasius* clade with poorly support (52%). The intrarelationships in the clade containing *Pangasius*, *Helicophagus* and *Pseudolais* were almost unresolved since the uniting branches collapsed into polytomies. *Pangasianodon* was still as monophyletic group and as basal taxon. Within schilbeid clade, the MP analysis gave the identical topology to NJ phylogeny with the placement of *Pseudeutropius* at the basal position (78% of bootstrap support value).

ML phylogeny using the RNA data set demonstrated in Figure 18. The ML analysis yielded a strange topology which differed from NJ and MP-based RNA analyses as well as the *cyt b* phylogeny in which the schilbeid genus *Pseudeutropius* changed to the pangasiid clade at the basal position but with poorly support (58%). However, all of members of Pangasiidae were still monophyletically grouped with highly statistical confidence (97%) with the recognition of *Pangasianodon* as basal lineage. *Pangasius* was still recovered as polyphyletic assemblage including *Helicophagus* and *Pseudolais.* Within schilbeid clade, Thai schilbeids were monophyletically grouped with 100% of statistical confidence. *C. sinense* was grouped with *L. longibarbis*. Unlike the results found in NJ and MP analyses, *C. prateri* had a trend to cluster with *E. Salweenensis* but with only 61% of bootstarp support value.

The BI-based RNA phylogeny as shown in Figure 19 is similar to the ML tree. The *Pangasius* was still recovered as polyphyletic assemblage. The schilbeid genus *Pseudeutropius* appeared at the basal position of pangasiid clade but with poorly statistical support (60%). All of pangasiid taxa were still recognized as monophyletic with 100% of statistical confidence. The intrarelationships within this clade were largely unresolved. *Pangasianodon* embeded within *Pangasius* but with poorly statistical support (54%). *Helicophagus* was sister taxon of a clade consisting of *Pangasius* and *Pseudolais* was recognized as the basal taxon of all pangasiids. Within schilbeid clade, the reconstructed BI tree revealed the identical topology as found in ML tree. *C. sinensis* was well aggregated (100% of bootstrap) with *L. longibarbis*, while *C. prateri* was grouped with *E. salweenensis* with poorly statistical support value (68%).

In the present study, phylogenetic analyses based on the RNA data set using NJ, MP, ML and BI analyses provided the poor resolution compared with the phylogeny-based *cyt b* gene. RNA phylogenies contributed the weak statistical support value for the familial level (66%-100% support values at 6 interior branches from NJ, MP, ML and BI trees, Table 17). The less resolved trees of RNA analysis might be due to the relative effects of genetic marker. Different genes or genomic

regions possess the different patterns of evolution (Kocher and Carleton, 1997) that might be appropriate for resolving a particular phylogenetic question among the organisms at a certain taxonomic levels. The highly conserved molecular markers are useful for investigating phylogenetic relationships at higher taxonomic levels. On the other hand, the hypervariable molecular markers are useful for elucidating the relationships at low taxonomic levels (Hwang and Kim, 1999). In present study, the RNA data set contained only 289 potentially informative characters (16%) of all 1,850 characters. This paucity of informative characters reflects the fact that mt *rRNA* evolves slower than mt protein coding genes and fail to provide significant phylogenetic results at generic levels (Crow *et al*., 2004; Moyer *et al*., 2004; Orrell and Carpenter, 2004). As seen in this study, the phylogenetic analyse based on RNA data did not provide deep phylogenetic relationships between genera of Pangasiidae. In addtion, indel characters of the mt *rRNA* sequences might limit for phylogenetic approaches (Crow *et al*., 2004). In present study, there were 56 portions of indels and 312 gaps identified in the alignment of the RNA sequences. Although all of gaps were excluded from the analysis in this study, the tree topologies were still largely unresolved. Lutzoni *et al*. (2000) concluded that if the ambiguously aligned regions are included in phylogenetic analyses, the homologies are likely to violate and phylogenetic accuracy might be lowered considerably. If excluded, however, the resolving power may be jeopardized. Recognizing these problems, several authors (Page and Holmes, 1998; Lutzoni *et al*., 2000; Hall, 2001) have proposed that modification of gap penalties used by the computer program is probably the single most important thing that can do to improve the alignment and make a high quality phylogeny possible. In addition, the unexpected result was found in ML and BI trees in which the schilbeid genus *Pseudeutropius* was placed to pangasiid clade but with poorly supports (58% and 60%) compared with those at recovered in NJ and MP trees. Thus, *Pseudeutropius* should be positioned as the basal lineage in the family Schilbeidae. The incongruence topologies might correlate to the large indel variation in the RNA data set which could violate the positional homology of the alignments (Lutzoni *et al*., 2000). The unexpected results in ML and BI analyses might not correct topologies because the accuracy of ML and BI-based phylogenies is very dependent on the right of the positional homology at all nucleotide sites rather than NJ

and MP methods which the distance matrix and only the informative sites are used for phylogenetic analyses, respectively (Hall, 2001). Within the pangasiid clade, all phylogenies (NJ, MP, ML and BI analyses) inferred from the RNA data set highly supported the monophyletic relationships of *Pangasianodon*, *Helicophagus* and *Pseudolais* but polyphyletic in *Pangasius*. The placement of *Pangasianodon* at the basal position of Pangasiidae was clearly evidenced in almost analyses (NJ, MP and ML trees). The interrelationships among *Pangasius*, *Helicophagus* and *Pseudolais* were unresolved in the majority of phylogenetic analyses inferred from the RNA data set (NJ, MP and ML trees), indicating that the RNA data have no enough variation to resolve the relationships among pangasiids at the generic level, therefore, more samplings of nucleotide characters from the other genetic loci should provide more resolved phylogeny.

Within schilbeid clade, all topologies highly supported the polyphyletic assemblage of the genus *Clupisoma*. *C. sinense* was well aggregated with *L. longibarbis* in all analyses, supporting that the former taxon should belong to *Laides* as described by Kottelat (1989); Zakaria-Ismail (1992) and Rainboth (1996). *C. prateri* was recovered as sister to *C. sinense* + *L. longibarbis* in NJ and MP trees with 85% and 82% of bootstrap values, respectively, but changed to sister to *E. salweenensis* in ML and BI topologies with the lower statistical confidences (61% and 68%, respectively). Although the close affiliation of *C. prateri* and *E. salweenensis* was found, however, these two lineages are morphologically diverse and were recognized as separate genera by several previous studies (Vidthayanon and Roongthongbaisuree, 1993; Rainboth, 1996; Ferraris, 2007; Ferraris and Vari, 2007). They have marked differences in the cleft of the mouth and palatal tooth patches (oblique and extending to the front border of the eyes in *E. salweenensis*; Ferraris and Vari, 2007 vs. not oblique and not extending to front edge of eyes in *C. prateri*; Ferraris, 2004). The palatal teeth of *E. salweenensis* arrange in a broadly parabolic patches (Ferraris and Vari, 2007), whereas *C. prateri* possesses two separate elongated ovoid palatal tooth patches (Ferraris, 2004).

To assess the phylogenetic position of the putative new schilbeid species, *E. salweenensis* previously recognized as *E. vacha* (Ferraris and Vari, 2007), the available partial sequence (465 bp) of *16S rRNA* of *E. vacha* from GenBank (GQ357917) was also included for inferring phylogenetic relationships. Because the *16S rRNA* sequence of *E. vacha* is relatively short (465 bp) compared with the RNA data set in this study (1,850 bp), thus all of the RNA sequences from this study were trimmed to the size of the smallest fragment (465 bp) to maintain consistency of the data. Then this trimmed *16S rRNA* data set was used to analyze phylogenetically with the analogous methods (NJ, MP, ML and BI). The obtained *16S rRNA* trees were similar in all methods and the phylogeny is shown in Figure 20. The phylogeny demonstrated that *E. salweenensis* is positioned in schilbeid clade and is recovered as sister to its congener, *E. vacha* with 100% of statistical confidence. The interspecific *p*-distance between *E. salweenensis* and *E. vacha* was 2.2% which is consistent with the sequence divergence used to separate fish species in the Antarctic fish genus *Trematomus* (0.5% - 3.9%; Ritchie *et al*., 1996). Thus, the species status of *E. salweenensis* could be confirmed with genetic evidence from this study.

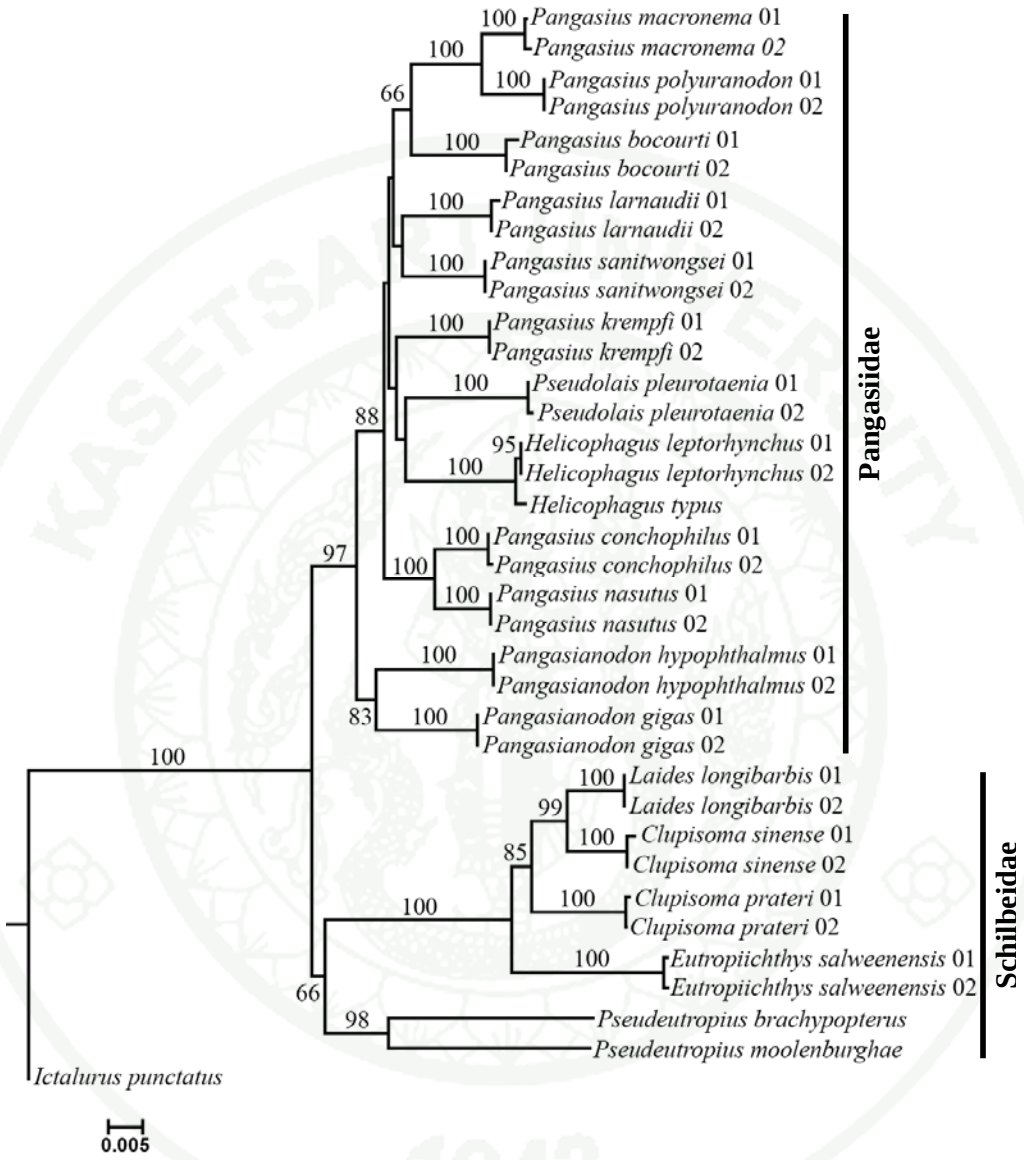


Figure 16 NJ tree of Thai pangasiids and schilbeids inferred from 1, 850 bp of RNA sequences. The scale bar represents 0.005 substitution/site. Numbers above branches represent bootstrap support for NJ (1000 replicates). Nodes with support value below 50% are not numbered.

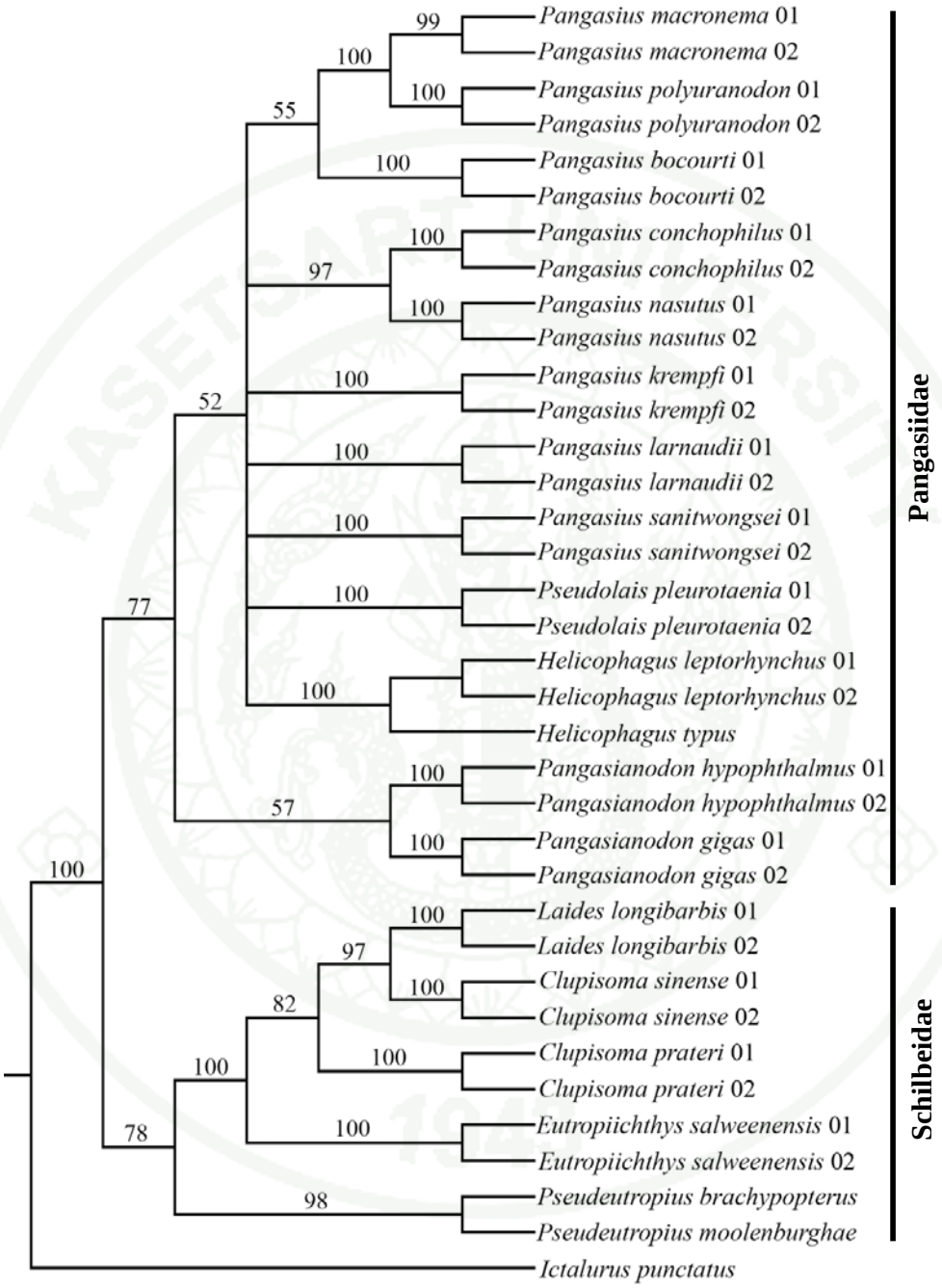


Figure 17 Consensus MP tree of Thai pangasiids and schilbeids inferred from 1,850 bp of RNA sequences. Numbers above branches represent bootstrap support (1000 replicates) for MP.

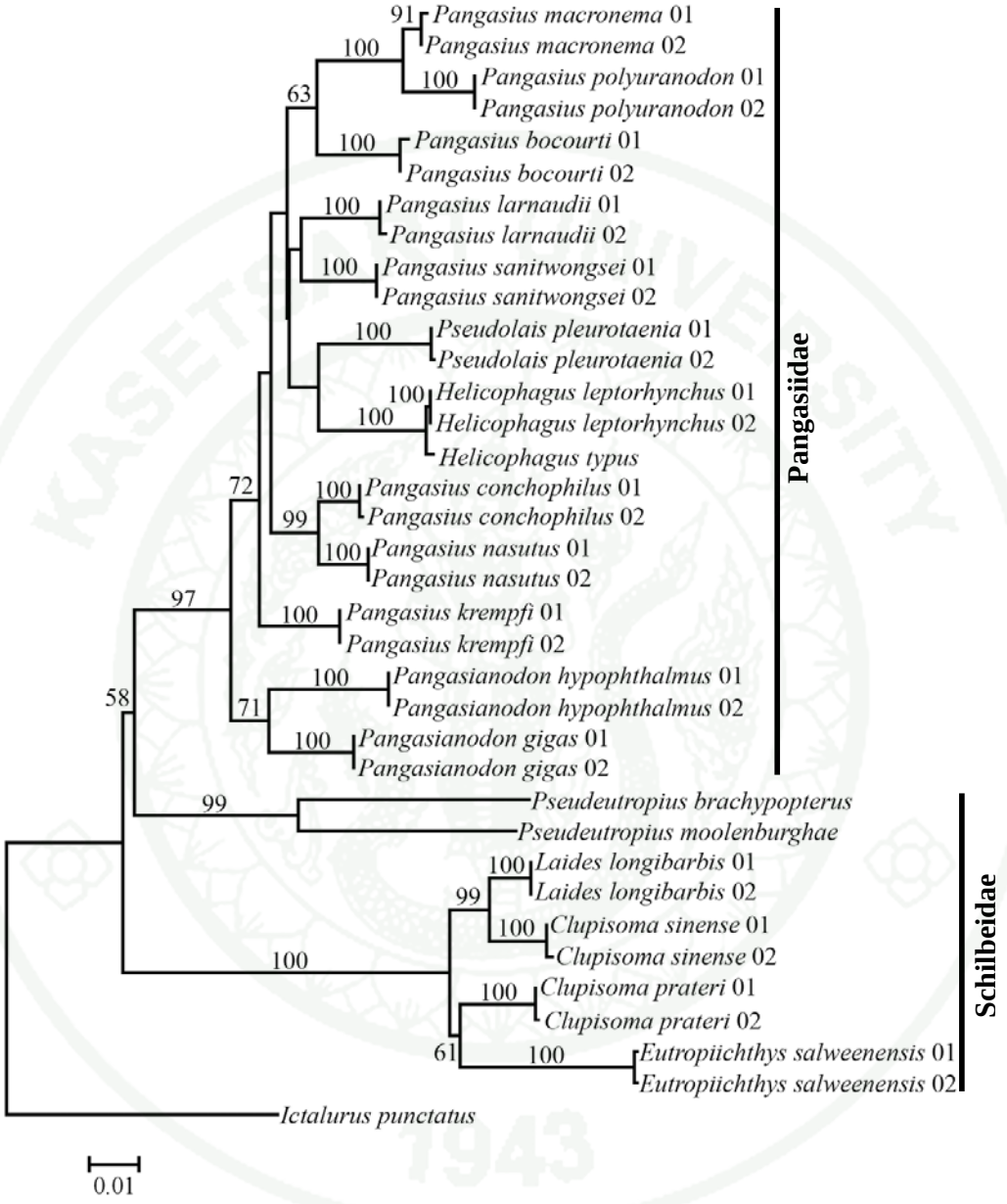


Figure 18 ML tree of Thai pangasiids and schilbeids inferred from 1,850 bp of RNA sequences. The scale bar represents 0.01 substitution/site. Numbers above branches represent bootstrap support for ML (500 replicates). Nodes with support value below 50% are not numbered.

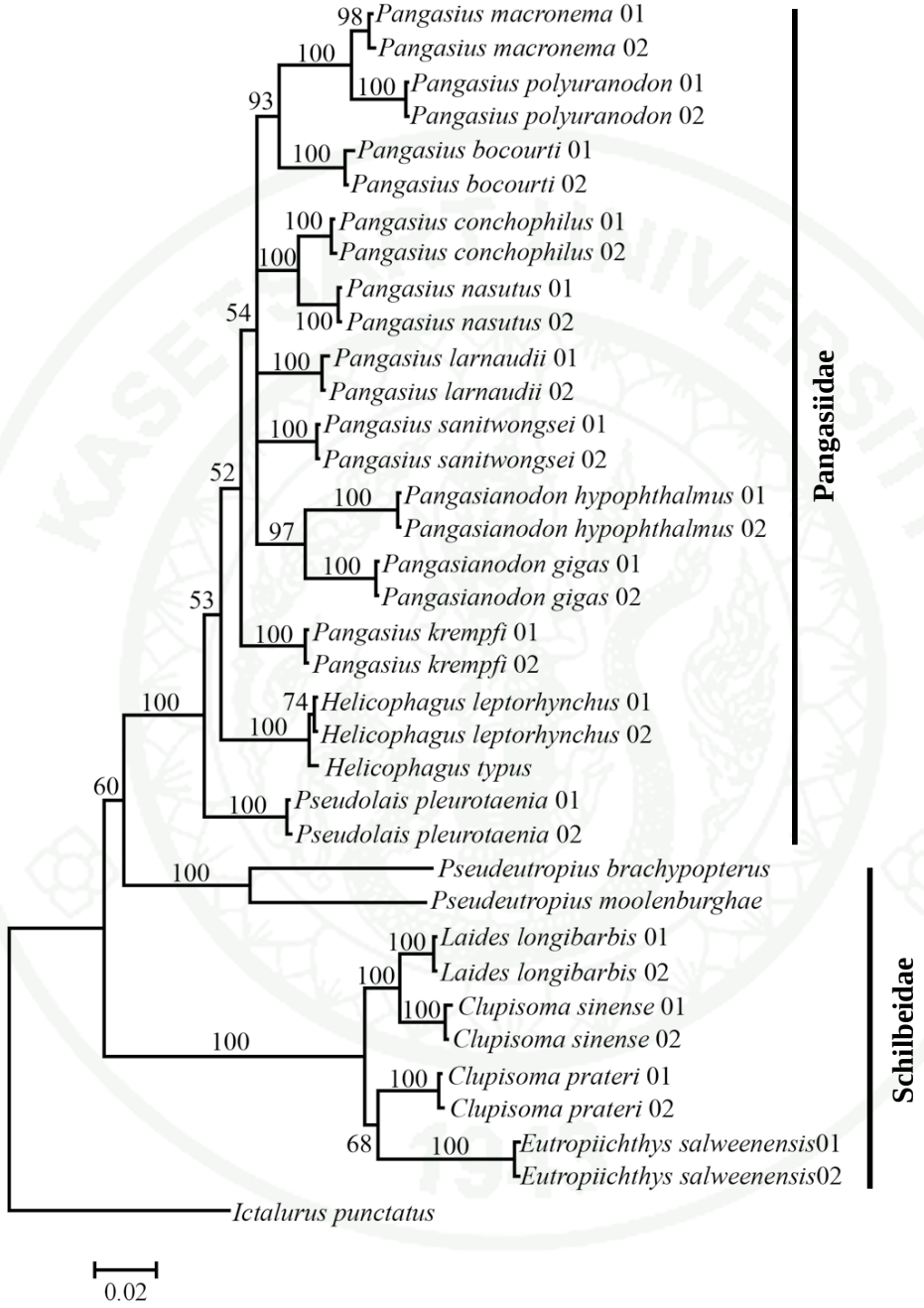


Figure 19 BI tree of Thai pangasiids and schilbeids inferred from 1,850 bp of RNA sequences. The scale bar represents 0.02 substitution/site. Numbers above branches represent the posterior probability percentage for BI.

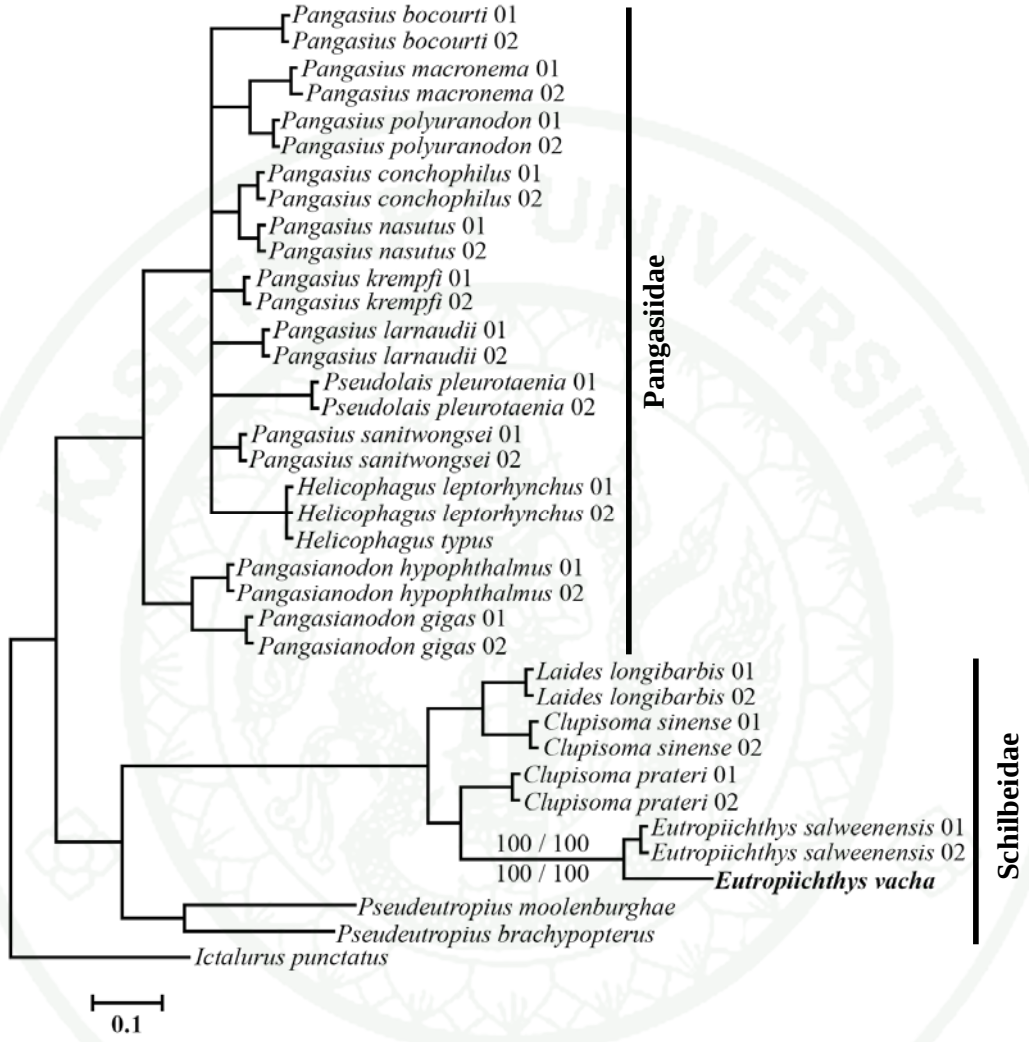


Figure 20 *16S rRNA* phylogeny of Thai pangasiids and schilbeids, focusing on the phylogenetic position of the new putative species, *Eutropiichthys salweenensis.* Names in regular font represent samples used in this study. The sequence of *E. vacha* from GenBank (GQ357917) was included and the species name represents in bold font. Numbers at the interior branch containing *E. salweenensis* and *E. vacha* represent support for NJ (upper left value), MP (upper right value), ML (lower left value) and BI (lower right value).

C. *RAG1* gene

The *RAG1* sequences of 35 taxa of pangasiids and schilbeids from this study and one outgroup species, *Ictalurus punctatus* (AF482987) were aligned. The RAG1 data set composed of 858 sites were used for phylogenetic analyses with the application of NJ, MP, ML and BI methods.

The phylogenetic topologies based on the nuclear *RAG1* data set shown in Figure 21-24. Overall topologies of NJ (Figure 21), MP (Figure 22), ML (Figure 23) and BI (Figure 24) trees appeared similar, therefore the phylogenetic results from NJ, MP, ML and BI analyses would be described together.

All phylogenetic trees based on *RAG1* gene highly supported (95%-100%) two main monophyletic lineages corresponding to the families Pangasidae and Schilbeidae. Within pangasiid clade, *RAG1* phylogenies clearly supported the monophyly of four pangasiid lineages corresponding to the genera *Pangasius, Helicophagus, Pseudolais* and *Pangasianodon* (Vidthayanon and Roongthongbaisuree, 1993). *Pangasius* was monophyletic group with 93%, 87%, 94% and 100% of statistical support in NJ, MP, ML and BI analyses, respectively. Compared with the trees generated from *cyt b* and RNA data sets, the nuclear *RAG1* gene was found to be the effective marker for classification at the familial and generic levels in which the statistical supports ranged from 87% to 100% (Table 17). Although *RAG1* data contained only 15% of informative characters (131 characters out of all 858 characters) which fewer than those found in *cyt b* (38%) and RNA (16%) data sets, however, there is no evidence for nucleotide saturation, even at third positions (Groth and Barrowclough, 1999; Martin, 1999). This is illustrated by the plots of nucleotide saturation test in this study. The *RAG1* regression lines (Figure 11) were more straighted than the regression lines observed in *cyt b* data set (Figure 9). In addition, indel variation did not found in *RAG1* data which is contrast to the RNA data. These properties make *RAG1* gene is more effective than *cyt b* and RNA data sets. However, at the species level, *RAG1* gave the poor resolution at 63%-100% for support a clade containing individuals of each pangasiid species (Table 17). This

reflects the fact that substitution rate of *RAG1* is relatively slower than those of almost all mt genes, thus it has proven useful for inferring deep phylogenetic relationships (i.e. order, familial and generic levels) as evidenced by several previously studies (Groth and Barrowclough, 1999; Murphy *et al*., 2001; San Mauro *et al*., 2004). Within *Pangasius*, most of the branching orders of all analyses were unresolved, since the bootstrap values were poorly supported. However, the well-supported sister group relationship between *P. macronema* and *P. polyuranodon* was found in all trees and also between *P. larnaudii* and *P. bocourti* in NJ topology. The genus *Pseudolais* was sister taxon of *Pangasius* with <50%, 54%, 60% and 91% of statistical confidence in NJ, MP, ML and BI, respectively. The monophyletic *Helicophagus* was confirmed as sister to *Pangasius* and *Pseudolais* clade with 69%, 66%, 72% and 97% in NJ, MP, ML and BI trees, respectively. In all topologies (NJ, MP, ML and BI), *Pangasianodon* was still recognized as a basal taxon with robust statistical confidence (99%-100%).

In the schilbeid clade, all analyses (NJ, MP, ML and BI) inferred from *RAG1* sequences yielded the stable phylogenetic position of all taxa and this result is consistent with the phylogenies obtain from *cyt b* and RNA analyses. *Clupisoma* was still polyphyletic in which *C. sinense* was rather closely affiliated to *Laides longibarbis* with highly statistical values (94%, 95%, 94% and 100% in NJ, MP, ML trees, respectively) than to *C. prateri.* The latter displayed a trend to cluster with *Eutropiichthys salweenensis* with high statistical supports (83%, 90%, 93% and 98% in NJ, MP, ML and BI trees). *Pseudeutropius* branched off from the others and was placed at the base with strongly statistical confidence (96%, 95%, 96% and 99% in NJ, MP, ML and BI).

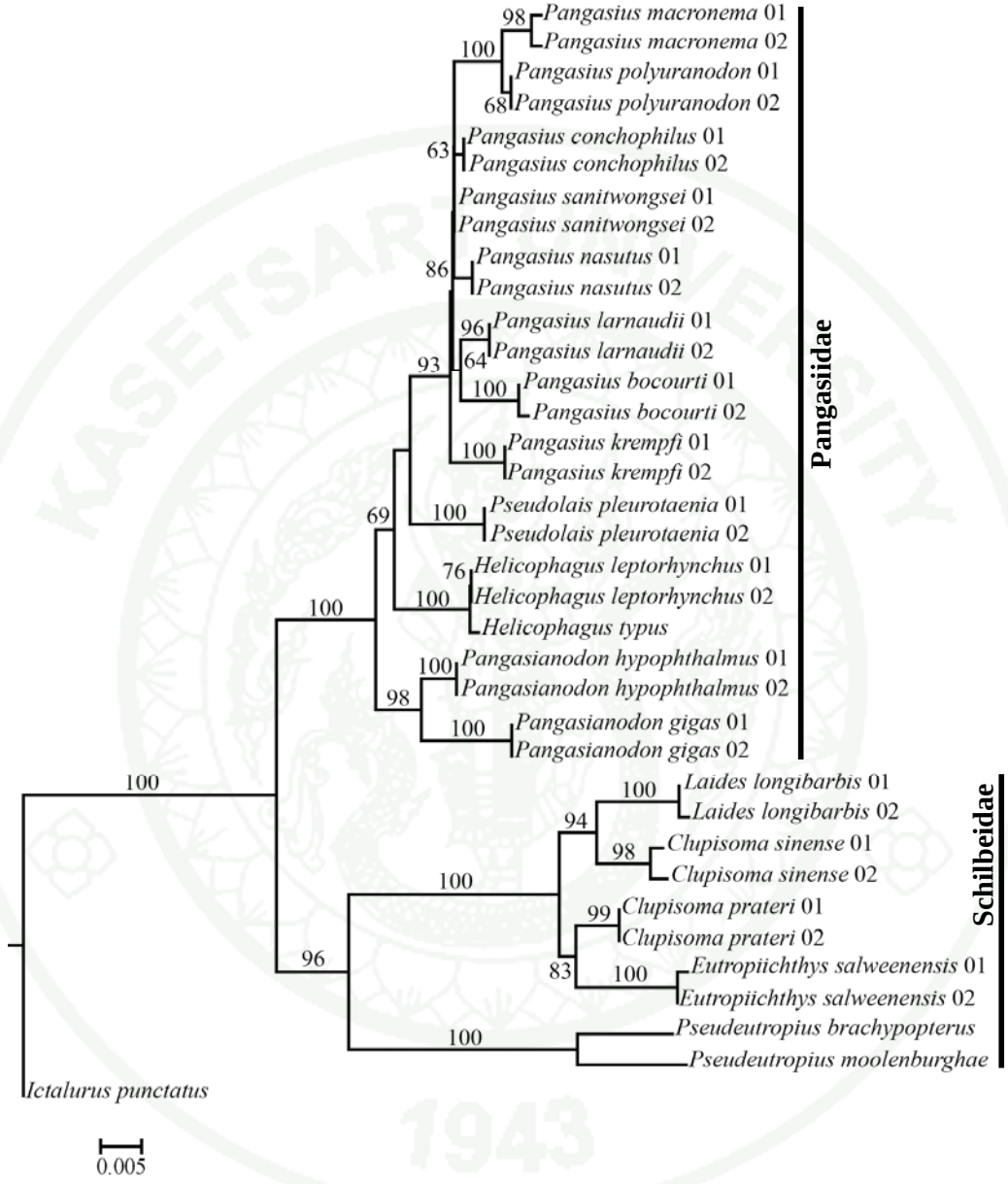


Figure 21 NJ tree of Thai pangasiids and schilbeids inferred from 858 bp of *RAG1* sequences. The scale bar represents 0.005 substitution/site. Numbers above branches represent bootstrap support for NJ (1000 replicates). Nodes with support value below 50% are not numbered.

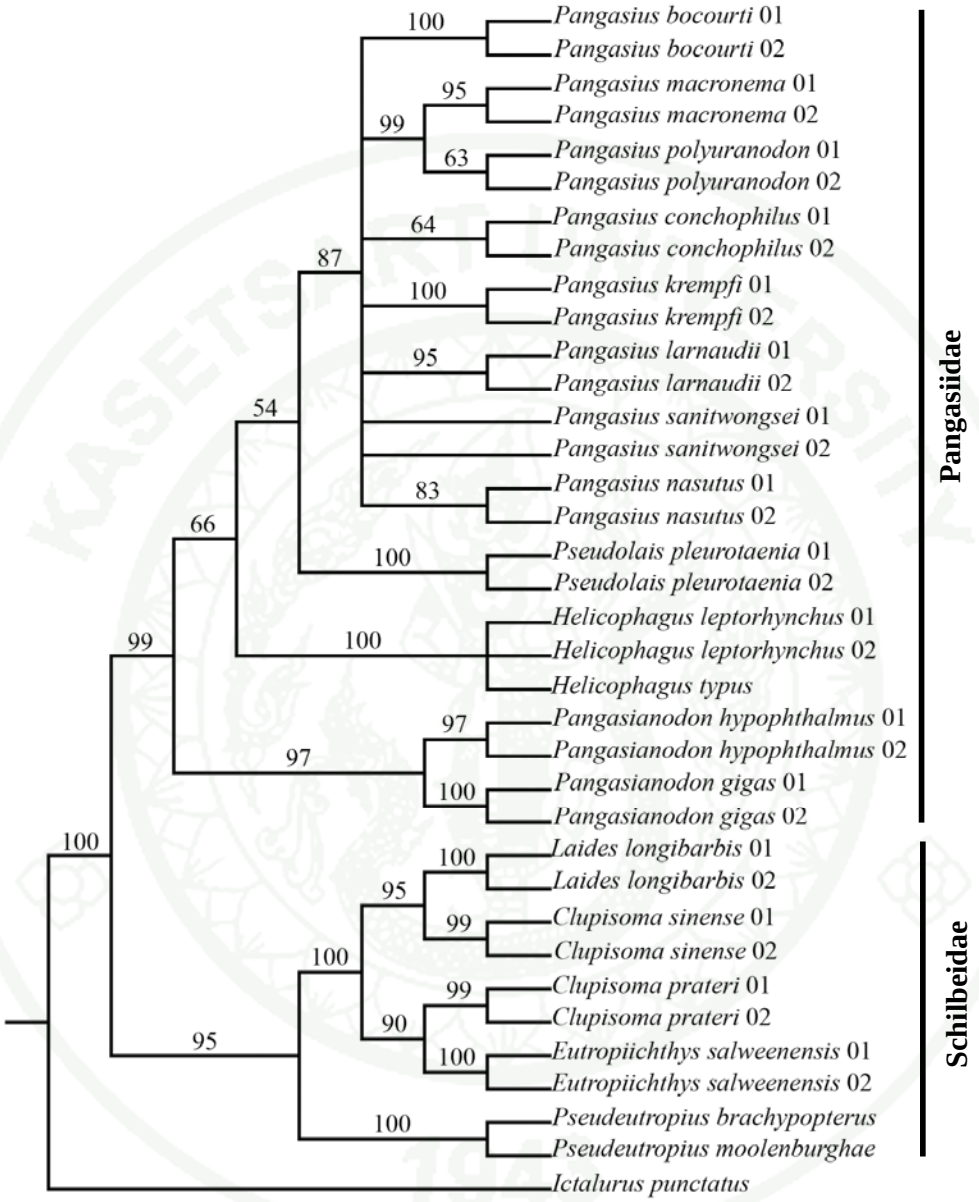


Figure 22 Consensus MP tree of Thai pangasiids and schilbeids inferred from 858 bp of *RAG1* sequences. Numbers above branches represent bootstrap support (1000 replicates) for MP.

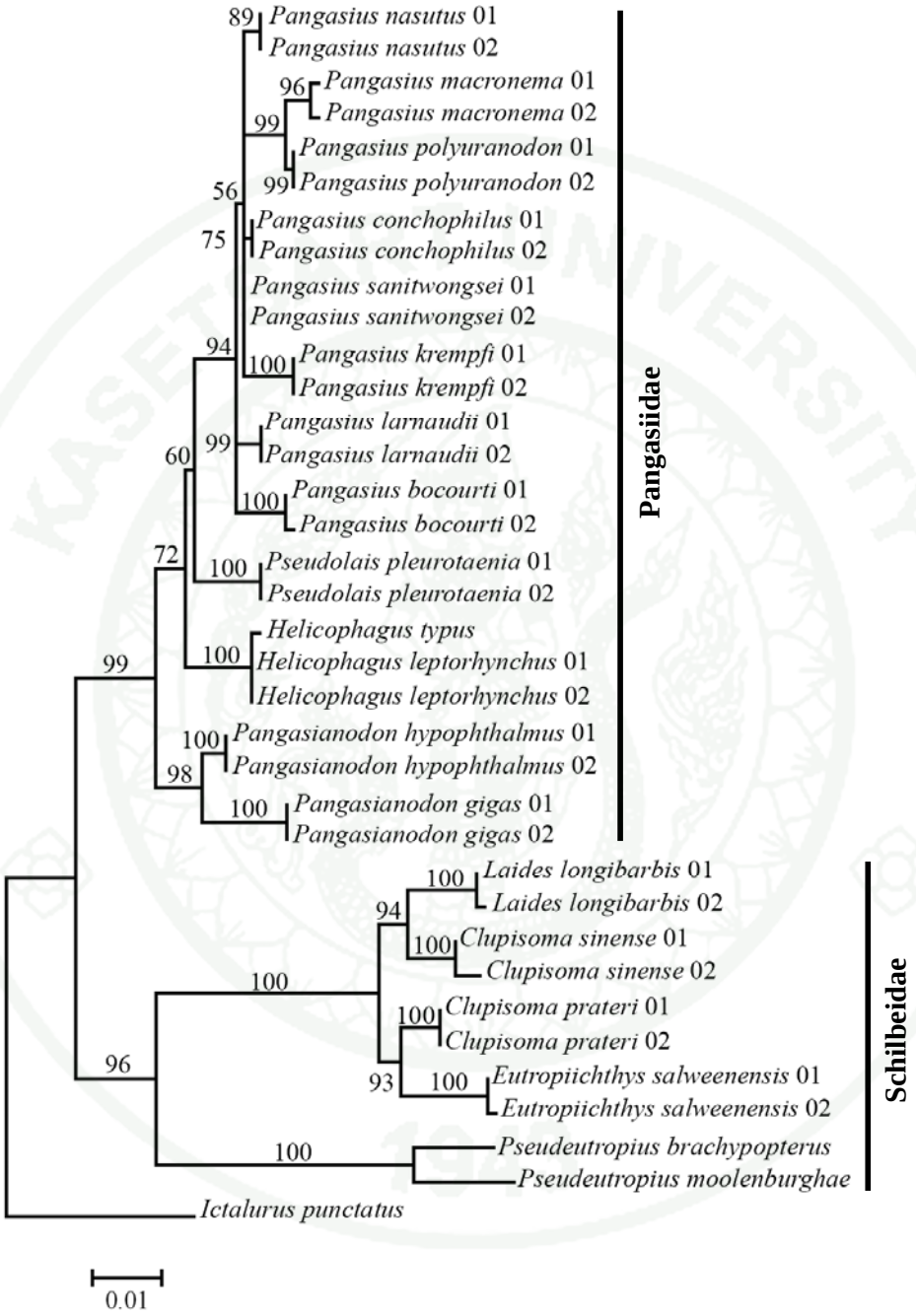


Figure 23 ML tree of Thai pangasiids and schilbeids inferred from 858 bp of *RAG1* sequences. The scale bar represents 0.01 substitution/site. Numbers above branches represent bootstrap support (500 replicates) for ML.

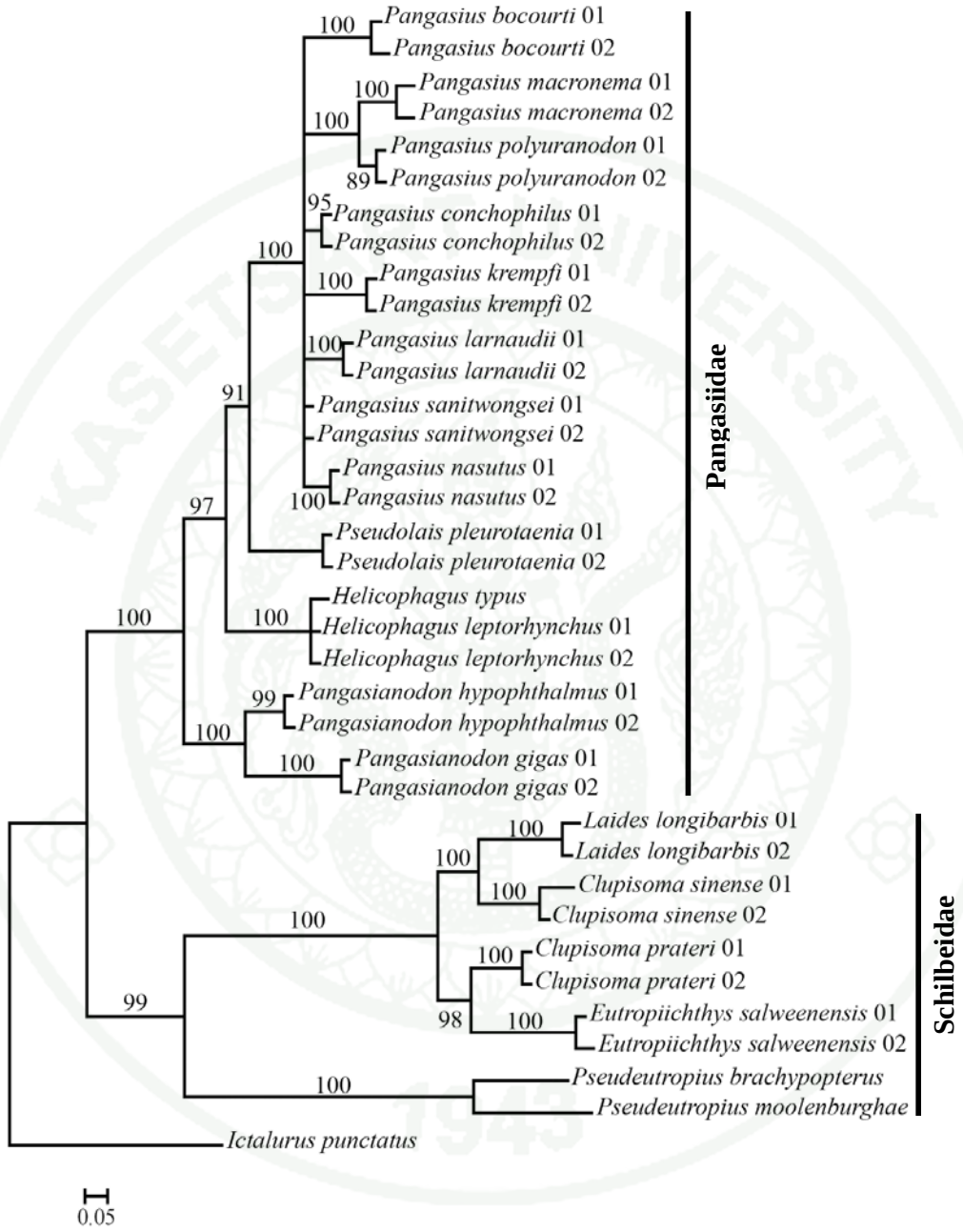


Figure 24 BI tree of Thai pangasiids and schilbeids inferred from 858 bp of *RAG1* sequences. The scale bar represents 0.05 substitution/site. Numbers above branches represent the posterior probability percentage for BI.

D. Combined mitochondrial and nuclear sequences

In recent years, several studies have demonstrated the need to establish phylogenetic inferences based on rather large sequence data sets in order to obtain well resolved phylogeny with highly statistical confidence (Cummings *et al*., 1995; Zardoya and Meyer, 1996; Rokas *et al*., 2003; Crow *et al*., 2004; Li *et al*., 2010). In this study, three genetic loci including the mt *cyt b* gene, the RNA fragment as well as the nuclear *RAG1* gene in total 3,814 bp with the parsimony informative sites increased to 837 characters were combined. The combined data set used to reconstruct phylogenetic relationships of all 35 taxa of pangasiids and schilbeids along with one outgroup species, *Ictalurus punctatus* (AF482987) with the application of NJ, MP, ML and BI methods.

The tree topologies based on the combined data set shown in Figure 25-28. Overall topologies of NJ (Figure 25), MP (Figure 26), ML (Figure 27) and BI (Figure 28) analyses are highly similar, therefore the phylogenetic results from NJ, MP, ML and BI analyses would be described together.

Based on the more sampling of sequences in the present study, NJ, MP, ML and BI phylogenies clearly provided the better resolved and better supported topologies (high statistical confidence) than the single-gene analyses both in the present (Table 17) and previous studies (Pouyaud *et al*., 2000; Pouyaud *et al*., 2004; Table 18). As evidenced by several previous studies (Cummings *et al*., 1995; Zardoya and Meyer, 1996; Rokas *et al*., 2003; Crow *et al*., 2004; Li *et al*., 2010), the concatenation of multiple gene sequences is known to increase the probability to obtain a robust phylogeny. All phylogenetic trees based on the combined data set demonstrated two district monophyletic clades corresponding to the families Pangasiidae and Schilbeidae with 100% of statistical confidence. Pangasiid clade was subdivided into three highly supported groups: *Pangasius*, *Pseudolais*+*Helicophagus* and *Pangasianodon*. All analyses-based the combined data gave the stable interrelationships among these groups, indicating that more variations in the combined data set are enough for resolving the relationships at the generic level. In

this study, *Pangasianodon* was recovered with high support (100%) as the most basal lineage in all trees. The next lineage that branched off the trees recovered *Pseudolais* and *Helicophagus* as sister group with moderate support. This clade was sister group of *Pangasius*, whose monophyly was highly supported in all trees (71%-100%). This result is inconsistent with previous molecular phylogenetic hypotheses based on allozyme and *cyt b* (Pouyaud *et al*., 2000) and *12S rRNA* data (Pouyaud *et al*., 2004). Pouyaud *et al*. (2000) proposed *Pseudolais* + *Helicophagus* as the basal group and *Pangasianodon* as more derived lineage, while Pouyaud *et al*. (2004) suggested *Pseudolais* as the most basal group and *Pangasianodon* as sister group to *Pangasius* + *Helicophagus*. Since using a large number of nucleotide should yield more resolved and more robust phylogeny (Cummings *et al*., 1995; Zardoya and Meyer, 1996), therefore, the novel phylogenetic hypothesis inferring from the combined data analyses in this study should reflect the more reliable evolutionary relationships of the family Pangasiidae. Within *Pangasius*, phylogenetic relationships among species were mostly unresolved, except the sister relationships of *P. conchophilus* + *P. nasutus* and *P. macronema* + *P. polyuranodon* with 100% of statistical support in all analyses. BI analyse contributed better resolved intrarelationships of *Pangasius* comprising three highly supported groups (Figure 28) as follows: group I, *P.macronema* associated with *P. polyuranodon* and *P. bocourti* as sister taxon of the clade; group II, *P. conchophilus* grouped with *P. nasutus* and *P. krempfi* as a sister taxon and finally group III, *P. larnaudii* associated with *P. sanitwongsei* and they were the basal lineage of the *Pangasius*. This result is concordant with the hypothesis proposed by Vidthayanon (1993) who suggested that *Pangasius* should be divided into three subgroups on the basis of snout, fin, swimbladder chamber and pelvic girdle characteristics. In the previous molecular phylogenetic studies of Pangasiidae (Pouyaud *et al*., 2000; Pouyaud *et al*., 2004), the inferred phylogenies did not allow to demonstrate of the possible subgenera in the genus *Pangasius*. The phylogenetic results from this study could indicate the existence of three possible sungenera in this genus.

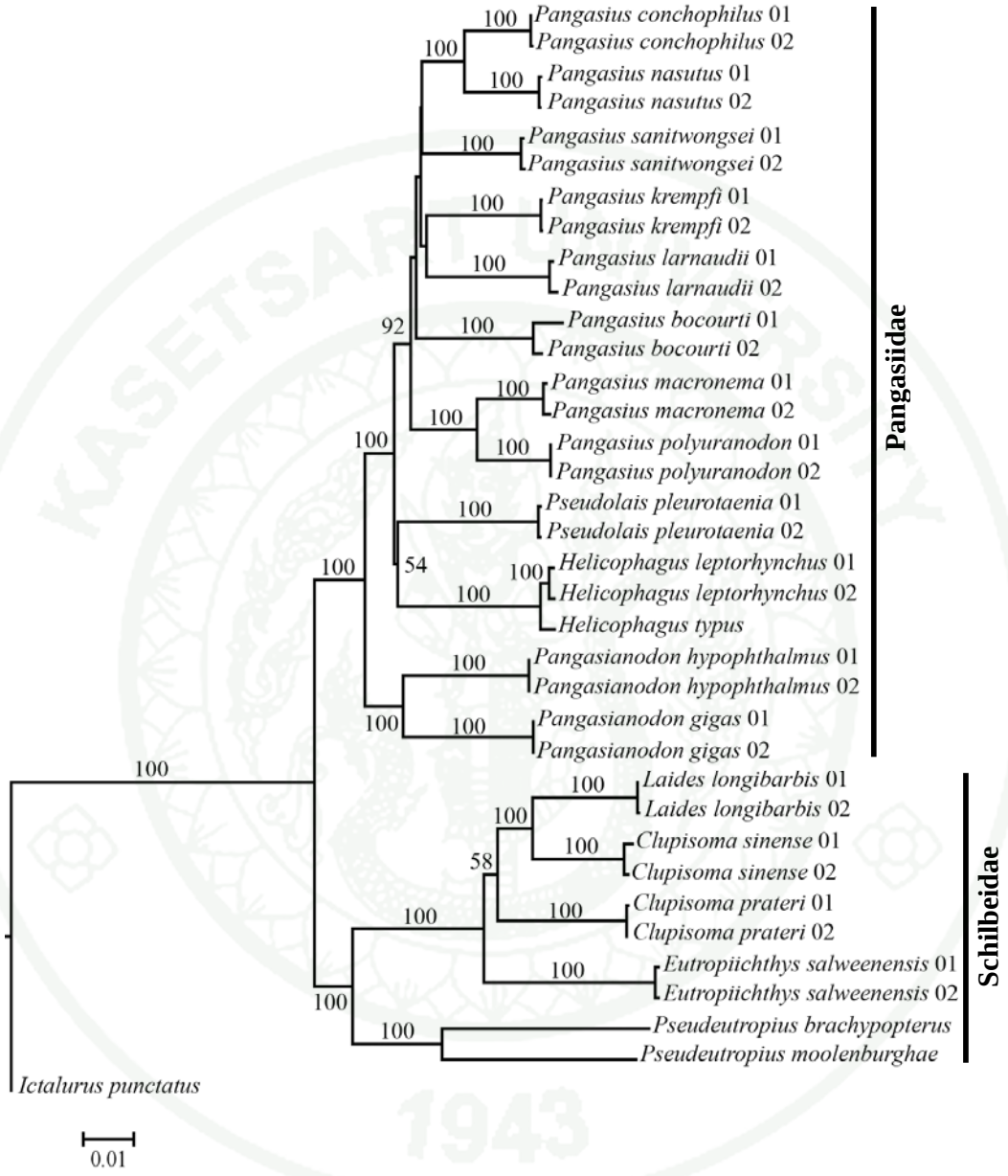


Figure 25 NJ tree of Thai pangasiids and schilbeids based on 3,814 bp of combined mt and nuclear sequences. The scale bar represents 0.01 substitution/site. Numbers above branches represent bootstrap support for NJ (1000 replicates). Nodes with support value below 50% are not numbered.

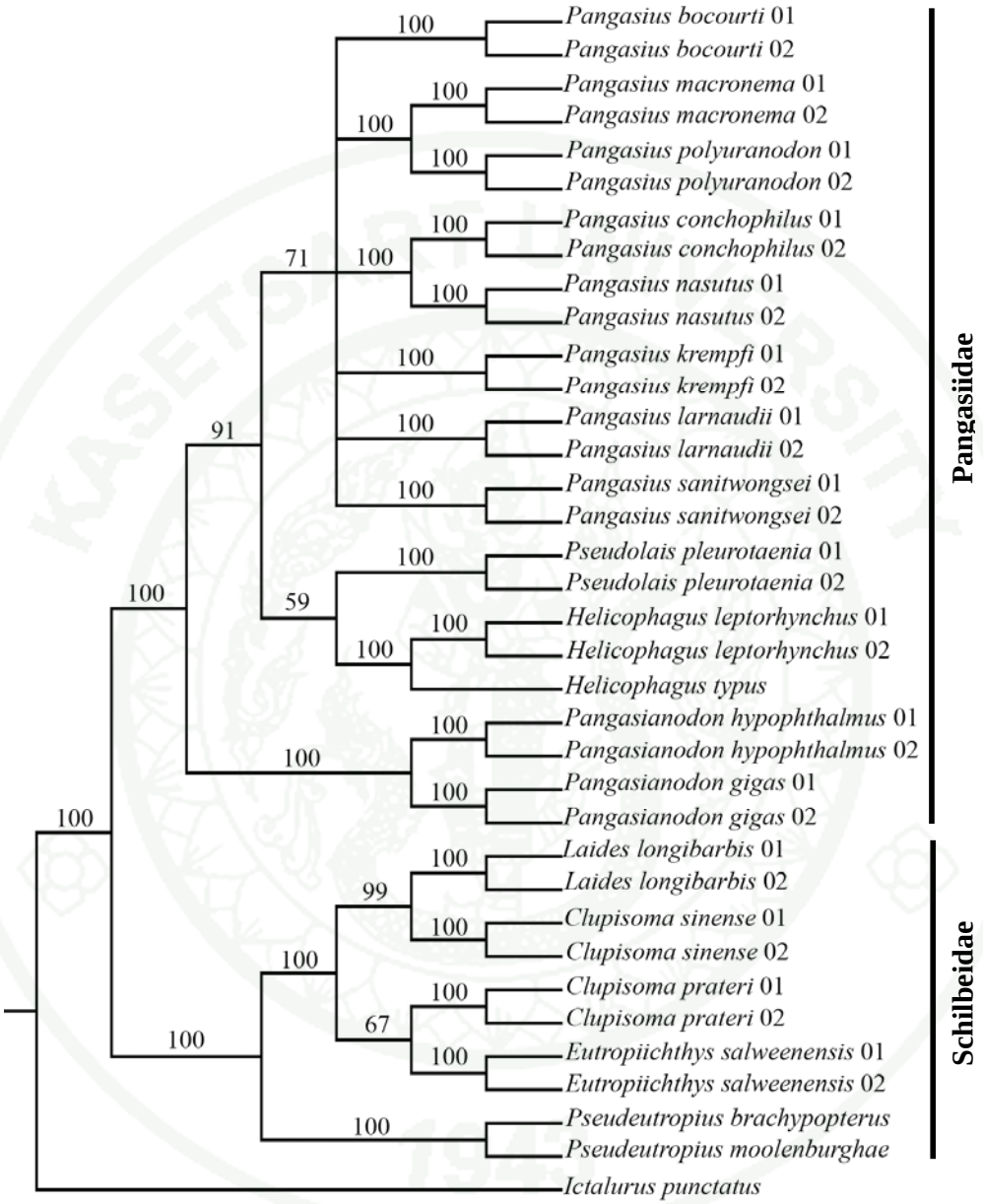


Figure 26 Consensus MP tree of Thai pangasiids and schilbeids based on 3,814 bp of combined mt and nuclear sequences. Numbers above branches represent bootstrap support (1000 replicates) for MP.

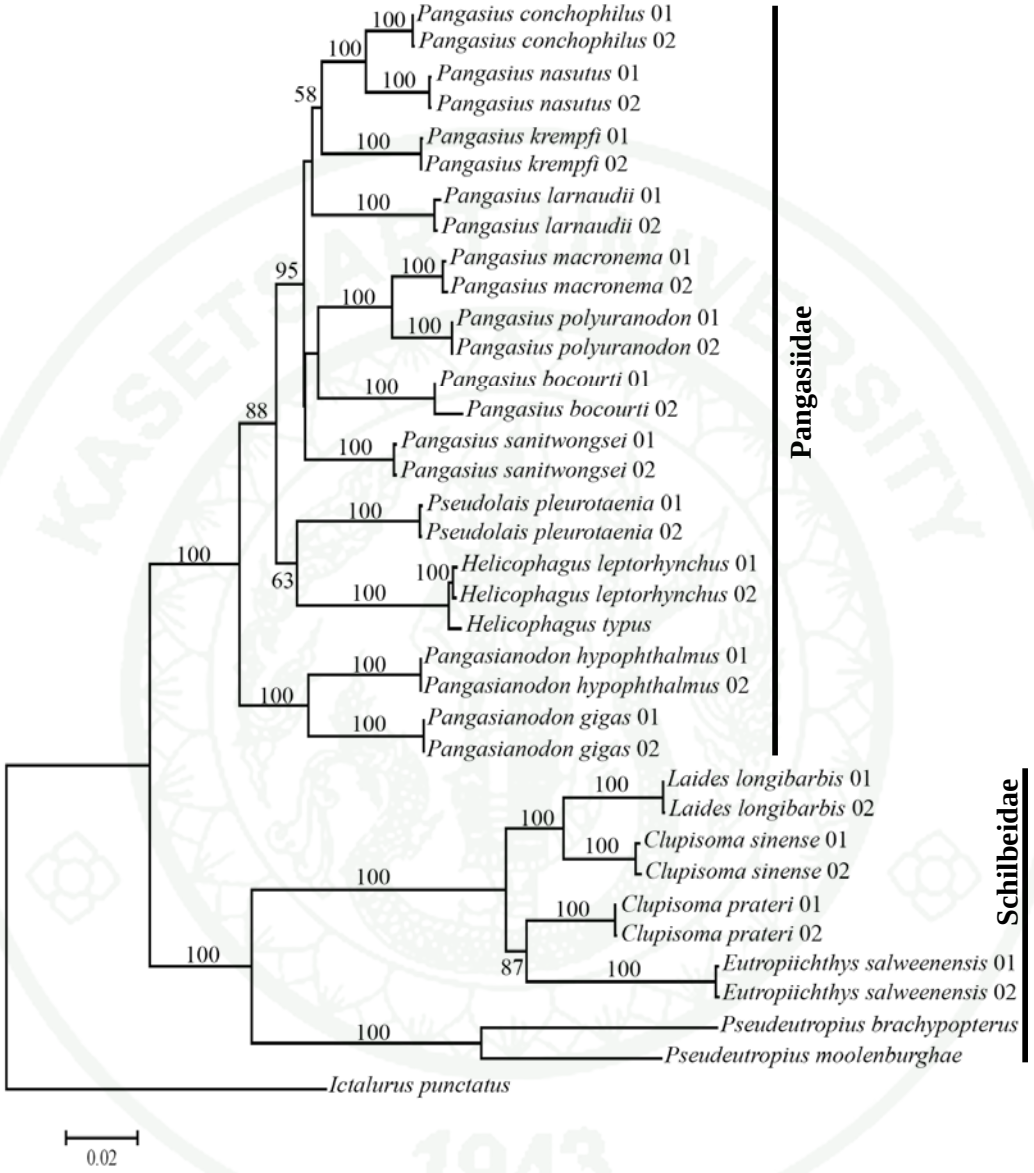


Figure 27 ML tree of Thai pangasiids and schilbeids based on 3,814 bp of combined mt and nuclear sequences. The scale bar represents 0.02 substitution/site. Numbers above branches represent bootstrap support (500 replicates) for ML. Nodes with support value below 50% are not numbered.

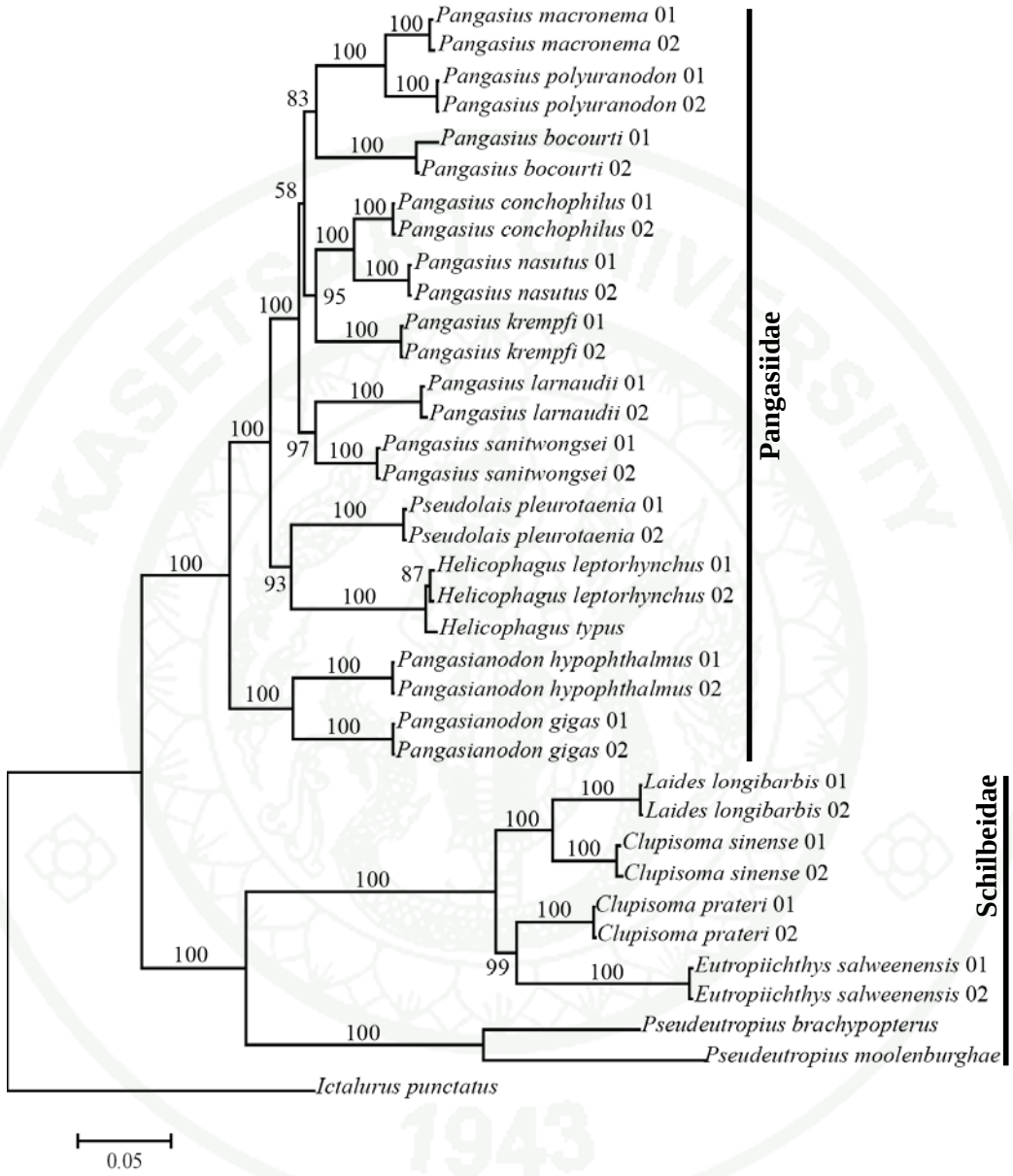


Figure 28 BI tree of Thai pangasiids and schilbeids based on 3,814 bp of combined mt and nuclear sequences. The scale bar represents 0.05 substitution/site. Numbers above branches represent the posterior probability percentage for BI. Nodes with support value below 50% are not numbered.

Regarding to the taxonomy of Pangasiidae, the generic classification of this family was still problematic in previous studies on the basis of morphological evidences. The number of recognized genera has been proposed varied from two to four. Based on the features of ethmoid region, mouth, premaxillary bones and vomerine toothband, several taxonomists (Roberts and Vidthayanon, 1991; Vidthayanon, 1993; Pouyaud *et al*., 1999; Teugels, 2003; Gustiano *et al*., 2004) classified pangasiids only into two genera, *Helicophagus* and *Pangasius*, and recognized *Pteropangasius* (or *Pseudolais*) and *Pangasianodon* as subgenera of the genus *Pangasius.* According to the characteristics of mouth, palatal toothband, swimbladder, abdomen and the number of fin rays, Vidthayanon and Roongthongbaisuree (1993) elevated *Pteropangasius* (or *Pseudolais*) and *Pangasianodon* to generic ranks, thus, four pangasiid genera including *Helicophagus, Pangasius*, *Pteropangasius* (or *Pseudolais*) and *Pangasianodon* were proposed. In contrast to previous studies, Rainboth (1996) considered the characteristics of barbels, the number of pelvic-fin rays and the location of posterior and anterior nostrils, suggesting three genera; *Pangasius*, *Helicophagus* and *Pangasianodon*, with the synonymization of *Pteropangasius* (or *Pseudolais*) with *Pangasius*. According to the phylogenetic results based on both single-gene and the combined sequences analyses in this study, *Pseudolais* and *Pangasianodon* clearly formed the independent clades branching off from *Pangasius*, therefore, they should not be synonym or subgenera of *Pangasius.* Although the close affiliation of *Helicophagus* and *Pseudolais* was found in the present study, these two lineages are morphologically diverse and were recognized as separate genera by several studies (Smith, 1945; Burgess, 1989; Robert and Vidthayanon, 1991; Vidthayanon, 1993; Pouyaud *et al*., 1999; Rainboth, 1996; Teugels, 2003; Gustiano *et al*., 2004). These lineages display marked differently in shape of mouth and snout, the number of swimbladder chambers (three chambers in *Helicophagus* and four in *Pseudolais*), and the palatal tooth patches, which are absent in *Helicophagus*. The reconstructed phylogenies together with the sequence divergences for each molecular marker suggest that there are four pangsiid genera including *Pangasius*, *Helicophagus*, *Pseudolais*, and *Pangasianodon.* This result is consistent with the morphologically taxonomic study as suggested by Vidthayanon

and Roongthongbaisuree (1993), who classified pangasiids with the characteristics of mouth, palatine tooth patch, swimbladder, abdomen and the number of fin rays.

Within schilbeid clade, NJ, MP, ML and BI trees inferring from the combined nucleotide sequences provided well-resolved and similar topologies as shown in Figure 25-28. The monophyletic schilbeid clade was subdivided into two groups. The first group contains *Laides longibarbis*, *Clupisoma sinense*, *C. prateri* and *Eutropiichthys salweenensis*. The other group comprising the two species; *Pseudeutropius brachypopterus* and *P. moolenburghae*, is at the basal position of schilbeid clade with 100% of statistical confidence in all analyses. Although the monophyletic relationships of Schilbeidae was presented in this study, the certain studies (Mo, 1991; Sullivan *et al*., 2006) with extensive taxon samplings of schilbeids and other catfish families placed the genus *Pseudeutropius* closer to other catfish family than to mostly Asian schilbeid genera (*Ailia* group in Mo, 1991). Based on the cladistic analysis of 126 morphological and anatomical characteristics, Mo (1991) recognized Schilbeidae as non-monophyletic and classified them into three groups: African and two Asian schilbeid groups. A '*Schilbe* group'representing the African schilbeids, '*Ailia* group' representing the Asian schilbeids such as *Ailia*, *Laides*, *Clupisoma* and *Eutropiichthys* and the third, '*Pseudeutropius* group' represented by another Asian schilbeids such as *Pseudeutropius*, *Platytropius* and *Horabagrus.* Recently, Sullivan *et al*. (2006) contributed molecular phylogeny of the major groups of catfishes by using *RAG1* and *RAG2* nuclear sequences and demonstrated that the genus *Pseudeutropius* grouped with the genus *Horabagrus*. These two genera closely relate to the family Bagridae rather than the Asian schilbeids including the genus *Ailia* and *Laides*. For further testing, the broader taxon sampling of schilbeids should be needed to confirm the taxonomic status of the genus *Pseudeutropius* as well as the monophyly of the family Schilbeidae. Graybeal (1998) suggested that the addition of taxa could be improved the accuracy of phylogenetic relationships rather than the addition of characters.

In *Clupisoma* group, the *C. sinense* well aggregated with *L. longibarbis* rather than *C. prateri.* A close phylogenetic relationship of *C. sinense* and *L.*

longibarbis is inconsistent with previous morphologically taxonomic studies proposed by Vidthayanon and Roongthongbaisuree (1993); Ng (1999); Ferraris (2004) and Chen *et al*. (2005) who suggested that *L. sinensis* ought to be classified as *C. sinense.* However, this result is in agreement with Kottelat (1989), Zakaria-Ismail (1992) and Rainboth (1996) who placed the *C. sinense* within the genus *Laides* as *L. sinensis* based on the morphology of the anterior and posterior nostrils, the palatal tooth patches, barbels shape, pelvic fin rays and pectoral fin spine. Therefore, the presence of the four pairs of barbels in *L. sinensis* which was used to place the species within *Clupisoma* (Vidthayanon and Roongthongbaisuree, 1993; Ng, 1999; Ferraris, 2004; Chen *et al*., 2005) seems to be a convergence. Although *C. sinense* is also superficially resemblant to *C. prateri* with four pairs of barbels and gas bladder greatly reduced, it possesses some divergent features from *C. prateri* including the contraction of the pectoral fin spine to pelvic fin origin (in contrast to the extension of the pectoral fin spine beyond the pelvic fin origin in *C. prateri*); six pelvic fin rays (conversely five); two small ovoid and oblique palatal tooth patches that nearly extend to the midline (vs. two separate elongated patches, ovoid, non attainment to the midline) (Ferraris, 2004; Chen *et al*., 2005). These characters of *C. sinense* are very resemblant to those in *L. longibarbis.* Moreover, *C. sinense* and *L. longibarbis* are commonly found in the Mekong River and have thus been mutually caught, whereas *C. prateri* only distributes in the Salween River (Vidthayanon and Roongthongbaisuree, 1993; Rainboth, 1996). As for the phylogenetic results, along with the morphological and the ecological evidence, this study agrees with previous taxonomic status proposed by Kottelat (1989), Zakaria-Ismail (1992) and Rainboth (1996) for the recognition of *L. sinensis*. On the phylogenetic position of *C. prateri*, it branched off from *L. longibarbis* and *C. sinense* and was placed as a sister taxon with 58% of statistical confidence in NJ tree (Figure 25) but changed to sister position of *E. salweenensis* in MP, ML and BI with 67%, 87% and 99%, respectively (Figure 26-28). The close affiliation of *C. prateri* and *E. salweenensis* was also found in the single-gene analyses. However, these two lineages have marked different characters, such as cleft of mouth and palatal tooth patches and they were recognized as separate genera by several previous studies (Vidthayanon and Roongthongbaisuree, 1993; Rainboth, 1996; Ferraris and Vari, 2007; Ferraris, 2007). The sequence divergence

(*p*-distance) between *C. prateri* and *E. salweenensis* was determined as 11.3% in *cyt b*, 4.0% in RNA and 1.8% in *RAG1*. These values were in line with mean sequence divergence between genera in other fishes. In *cyt b* gene, the average sequence divergence between genera within Poeciliidae (Doadrio *et al*., 2009) and Goodeidae (Doadrio and Domínguez, 2004) ranged from 8% to 11%. Mean intergeneric sequence divergence estimating from the combined *12S* and *16S rRNA* sequences between genera within hagfishes was 2.25% (Kuo *et al*., 2003). The average *RAG1* sequnece divergence between cichlid fishes genera *Caquetaia* and *Theraps* was 1.7% (Hulsey *et al*., 2010). Based on the phylogenetic approach together with sequence divergences, the results in this study confirm the recognition of three extant Thai schilbeid genera; *Laides*, *Clupisoma* and *Eutropiichthys* as described by Vidthayanon and Roongthongbaisuree (1993).

Table 17 Percentage of statistical support on NJ, MP, ML and BI trees of each molecular marker using for pangasiids and schilbeids taxonomic classification

Taxonomic levels		Statistical supports for each molecular marker (%)															
		Cyt b				RNA				*RAG1*				Combined data			
		NJ	**MP**	**ML**	**BI**	**NJ**	**MP**	**ML**	**BI**	**NJ**	**MP**	**ML**	**BI**	**NJ**	**MP**	**ML**	**BI**
Family	Pangasiidae	97	95	93	98	97	77	97	100	100	99	99	100	100	100	100	100
	Schilbeidae	96	90	100	100	66	78	(P)	(P)	96	95	96	99	100	100	100	100
Genus	*Pangasius*	64	(P)	<50	(P)	(P)	(P)	(P)	(P)	93	87	94	100	92	71	95	100
	Pseudolais	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Helicophagus	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Pangasianodon	99	98	98	100	83	57	71	97	98	97	98	100	100	100	100	100
	Laides	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Clupisoma	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)
	Eutropiichthys	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Pseudeutropius	100	88	100	100	98	98	99	100	100	100	100	100	100	100	100	100
Species (16 species in total 16 subclades)		100 (16)	100 (15) 96(1)	100 (16)	100 (15) 98(1)	95(1) 100 (15)	81(1) 99(1) 100 (14)	91(1) 100 (15)	74(1) 98(1) 100 (14)	NO(1) 63(1) 68(1) 76(1) 86(1)	NO(2) 63(1) 64(1) 83(1) 95(2)	NO(1) 65(1) 75(1) 89(1) 96(1)	NO(2) 89(1) 95(1) 99(1) 100(11)	100 (16)	100 (16)	100 (16)	100 (16)

Table 17 (Continued)

Taxonomic levels	Statistical supports for each molecular marker (%)															
	Cyt b				RNA				*RAG1*				Combined data			
	NJ	MP	ML	BI	NJ	MP	ML	BI	NJ	MP	ML	BI	NJ	MP	ML	BI
Species (16 species in total 16 subclades)									96(1) 98(2) 99(1) 100(7)	97(1) 99(2) 100(6)	99(2) 100(9)					

Annotation: NO = can not be classified (unresolved), P = polyphyletic assemblage, Number in parenthesis at the species level represent the number of clades.

Table 18 Comparison of the statistical support (bootstrap or posterior probability percentage) for each pangasiid subclade from the combined sequence phylogeny in this study and the phylogeny from the previous studies

References	Subclade	Bootstrap or posterior probability percentage (%) for each phylogenetic inference method			
		NJ	MP	ML	BI
Pouyaud *et al*., (2000)	*Pangasius*	75	The methods were not analysed in the study.		
	Pangasianodon	80			
	Helicophagus	Only one species was analysed			
Pouyaud *et al*., (2004)	*Pangasius*	<50	The methods were not analysed in the study.		
	Pangasianodon	84			
	Helicophagus	80			
This study	*Pangasius*	92	71	95	100
	Pangasianodon	100	100	100	100
	Helicophagus	100	100	100	100

9. Estimation of divergence times

The divergence times between Pangasiidae and Schilbeidae and within these families were estimated utilizing a Bayesian approach in combination with knowledge of the fossil record of pangasiid genus *Cetopangasius* and the result was shown in a tree in Figure 29. According to the relaxed clock calibration, the average time divergence between pangasiids and schilbeids occurred approximately 13.21 million years before present (M B.P.) with the 95% HPD during Miocene epoch ranging from 19.45 to 6.97 M B.P. Within pangasiids, the separation between the most basal lineage, *Pangasianodon* and the rest occurred around 6.73 M B.P. during the upper Miocene. The split between *Pangasius* and *Helicophagus* + *Pseudolais* also occurred in the late Miocene (5.11 M B.P.). The average time of divergence between

Helicophagus and *Pseudolais* was 4.26 M B.P. (6.42-2.33 M B.P.) corresponding to the late Miocene to early Pleistocene. The separation of *Pangasius* species was more recent and its intense radiation initiated around 4.0 M B.P. (95% HPD: 5.83-2.15 M B.P.), and extended to 1.58 M B.P. (95% HPD: 2.55-0.69 M B.P.) corresponding to the late Miocene to the late Pleistocene. The unresolved intrarelationships within *Pangasius* might be caused by the rapid diversification in a period of time and might be too brief to allow for the accumulation of synapomorphies as also found in the American cichlids (Martin and Bermingham, 1998). The most recent divergence within pangasiids, between *H. leptorhynchus* and *H. typus*, was approximately 0.3 M B.P. during the late Pleistocene epoch.

Within schilbeids, the split between the basal group, *Pseudeutropius* and the rest occurred in the middle to late Miocene (10.76 M B.P.). The average time of divergence among genera *Laides*, *Clupisoma* and *Eutropiichthys* was 4.61-3.89 M B.P. in the middle Pliocene. The most recent divergence was found between *C. sinense* and *L. longibarbis* during early Pliocene to early Pleistocene (2.62 M B.P., 95% HPD: 4.29-1.10 M B.P.). The radiation among schilbeid genera was more ancient (10.76-3.89 M B.P.) than that found in pangasiids. This reflects that schilbeids are a very diverse group which widely distributes through southern Asia and Africa (De Vos, 1995). Although the estimation of divergence time between Asian and African schilbeids has not yet studied and there is no evidence to demonstrate that how the schilbeids radiated and migrated to their current habitats, the several previous works hypothesized that an African-Asian distribution in other fish families might correlate with ancient vicariance associated with plate-tectonic events. Estimated divergence time between the African and Asian cichlids (Vences *et al*., 2001), channids (Li *et al*., 2006) and notopterids (Inoue *et al*., 2009) dated back to the Cretaceous (140-65 M B.P.) corresponding to the geological estimate of time for separation of India-Madagascar from the African part of Gondwanaland. It seems possible that ancestors of Asian and African lineages vicariantly diverged in a part of Gondwanaland and the former migrated to Eurasia on the Indian subcontinent. After their disembarkation from the Indian subcontinent, they might have migrated to other

areas of South and Southeast Asia. This assumption may explain concerning the historical biogeography of the Schilbeidae.

For a long time, naturalists have recognized that changes in drainage basin morphology through the geological processes are important factors influencing patterns of biodiversity and distributions of the Ichthyofauna (Kottelat, 1989). In Southeast Asia, two major processes have substantial impacts on diversification of freshwater fishes; the tectonic activity during the Miocene-Pliocene and the sea level fluctuations during the Pleistocene epoch (Rainboth, 1991). Among pangasiids and schilbeids, the deep divergence among lineages radiated during the Miocene-Pliocene boundary. This was a time of historical changes drainage geomorphology in which several currently disjunct rivers: for instance, the Chao Phraya and the Mekong were formerly contiguous and subsequently were isolated by the mountains. These changes frequently occurred through the tectonic activity and might have led to the diversification of Southeast Asian freshwater fish fauna (Rainboth, 1996) and probably including pangasiids. Thai pangasiids mainly distribute in the two major separated river basins, the Chao Phraya and the Mekong. The presently pattern of distribution of pangasiids in these two rivers probably reflects the ever-connecting of these rivers. Rainboth (1996) and Brophy (2002) hypothesized that the Mekong River once ever connected to the Chao Phraya River through the mechanism of stream captures caused by the tectonic movement. It is likely that the growing Mekong Basin captured the headwater of the Yom River which is the tributary of the Chao Phraya in northern Thailand. The previous connection between the Chao Phraya and the Mekong Rivers is also supported by overlapping distributions of fauna across these two rivers (Rainboth, 1996). Subsequently, forces from the uplift of the Himalayas caused movement along the ancient sutures in this area, with the mountain building throughout northern Thailand, resulting in the currently disjunct the Chao Phraya and the Mekong Rivers (Rainboth, 1996). This mechanism of drainage change perhaps may also have acted to isolate populations across ancestral species ranges in formerly connection area, facilitating the species diversification of pangasiids. This assumption was also used to explain the vicariant speciation in the cyprinids genus *Hypsibarbus* (Rainboth, 1996).

In schilbeids, the species are found in the Mekong (*Laides longibarbis* and *Clupisoma sinense*) and the Salween Rivers (*C. prateri* and *Eutropiichthys salweenensis*) were separated in early Pliocene. This time was consistent with the historical tectonic uplift of the Tibetan Plateau where the source of the Mekong and the Salween Rivers (Clark *et al*., 2004). And recently, the regional drainage history in southeastern Tibet suggested that the modern rivers draining in the plateau margin were once tributaries of a single, southward-flowing system which drained into the South China Sea (Clark *et al*., 2004). In the three uplifts of the plateau around Miocene-Pliocene boundary, the water system of this region was separated (He *et al*., 2001). Clark *et al*. (2004) proposed that the course of the Salween may have been captured and the original river course into the Mekong was isolated by the uplift of Moinigkawagarbo Mountain. This vicariant hypothesis might use to explain the diversification of schilbeids in this region in which their ancestor had a wide distribution at early stage of the uplift. Along with the uplift, the widely occurred ancestor gradually located in different basin and developed as the different species. This hypothesis was also used to describe the vicariant speciation of glyptosternoid fishes (He *et al*., 2001; Peng *et al*., 2006) and also cyprinid fishes in the genus *Schizothorax* (He and Chen, 2007) in the Tibetan Plateau region.

The mutually exclusive distributions of the sister species, *Pangasius conchophilus* and *P. nasutus* as well as *Helicophagus leptorhynchus* and *H. typus* might indicate a vicariance event, occurring in Pleistocene epoch. In the present, *P. conchophilus* and *H. leptorhynchus* are found from the Chao Phraya and the Mekong Rivers in mainland Southeast Asia, whereas their sister species, *P. nasutus* and *H. typus*, respectively, are known only in the Kapuas River in Borneo. The separation of these sister species might have correlated with the change of river configurations resulting from the development of extended rivers basins during periods of sea-level retreat in the Pleistocene (Rainboth, 1996). In the middle Pleistocene, during periods of maximum glaciations, sea level lowerings might have reached 150-160 m, sufficient to expose the Sunda Shelf as a subaerial land mass connecting Borneo, Sumatra, Java and mainland Southeast Asia (Bornbusch and Lundberg, 1989). This land mass was drained by several major river systems, at least by two rivers; the

North Sunda River draining in parts of western Borneo, eastern Sumatra and the eastern slopes of the Malay Peninsula and the South Indo-China River draining in parts of Sundaland south of the present-day Mekong delta and emptying into the South China Sea (Bornbusch and Lundberg, 1989; Voris, 2000). Post-Pleistocene, sea level rises and/or lowering of the sea bottom inundated the Sunda Shelf and separated these two river systems and fragmented each into present-day isolated branches. The Kapuas (Borneo) and Musi and Batanghari (Sumatra) Rivers are remnants of the North Sunda River. The Mekong delta may represent a portion of the South Indo-China River shifted northwards (Bornbusch and Lundberg, 1989). This hypothesis was widely used to explain the vicariant speciation in many Southeast Asian freshwater fish groups (Rainboth, 1991). This is illustrated by the allopatric distributions of the silurid sister species; *Hemisilurus mekongensis* and *H. moolenburghi* (Bornbusch and Lundberg, 1989), *Kryptopterus geminus* and *K. cryptopterus* (Ng, 2003) and *Wallago micropogon* and *W. leerii* (Ng, 2004) as well as the bagrid sister species between *Bagrichthys majusculus* and *B. macracanthus* (Ng, 2002). The former sister pair confines to the continental section of Southeast Asia (the Chao Phraya and the Mekong Rivers), while the latter distributes only in the insular section (Borneo, Sumatra and Java). Moreover, the overlapping distributions of contemporary pangasiid species which mostly distribute in both the Chao Phraya and the Mekong Rivers might also have been the product of expanded freshwater connections in the Pleistocene epoch.

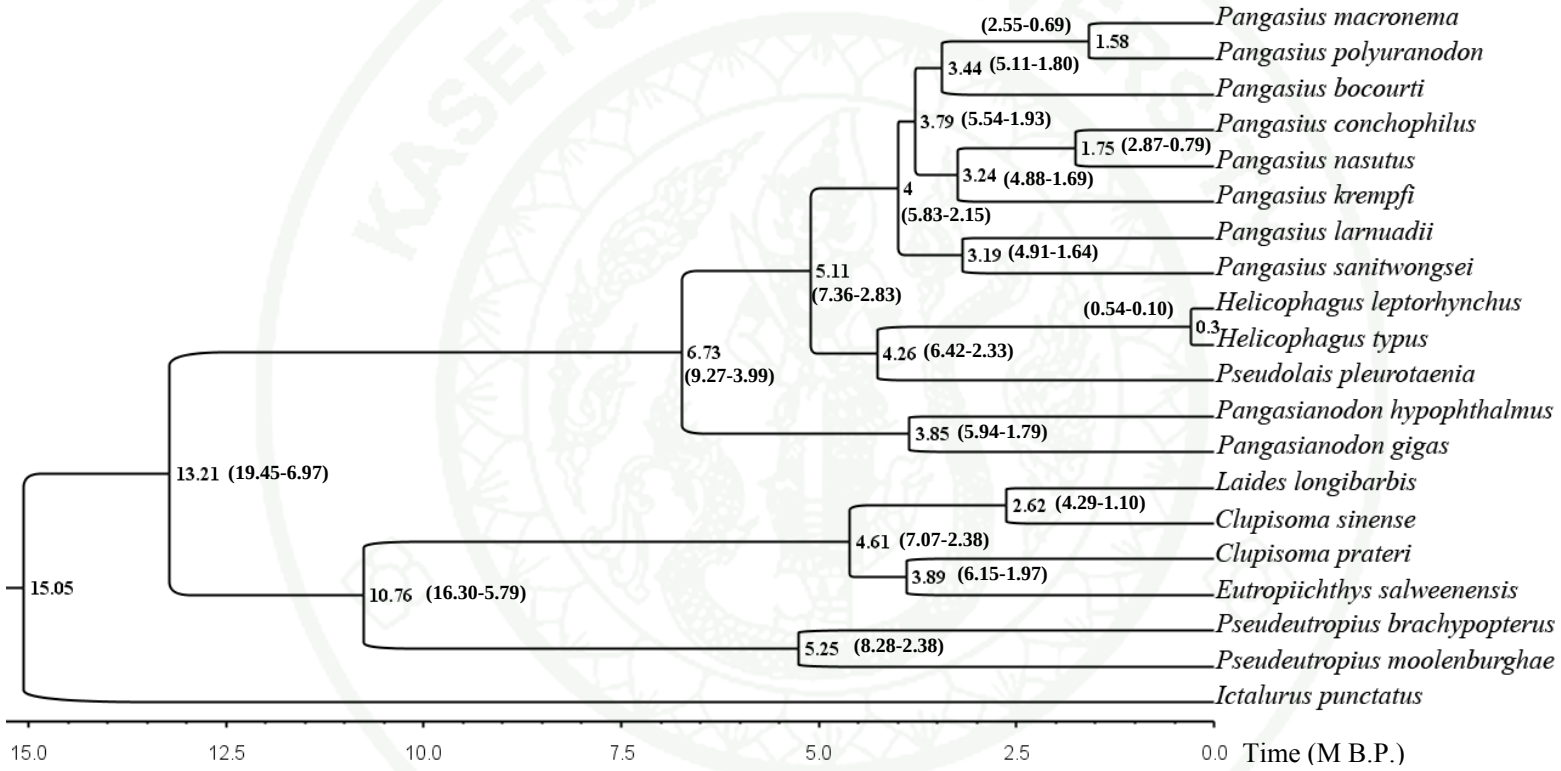


Figure 29 BEAST v.1.6.1 Bayesian tree showing the divergence times between Pangasiidae and Schilbeidae and within both families. Numbers at node represent mean age values in million years and the range of age (within parentheses). Time is shown in million years before present (M B.P.).

CONCLUSION AND RECOMMENDATION

Conclusion

Molecular phylogenies based on three molecular loci (*cyt b*, RNA and *RAG1*) clearly recovered the families Pangasiidae and Schilbeidae as monophyletic assemblage. Within the family Pangasiidae, four monophyletic subclades including *Pangasius*, *Pseudolais*, *Helicophagus* and *Pangasianodon* were recognized with high statistical supports. The *Pangasianodon* was strongly supported as the most basal taxon within pangasiids, whereas *Pseudolais* + *Helicophagus* were recovered as sister group of *Pangasius* which was recovered as the most recent diverged group. In combination with the values of sequence divergence (*p*-distance) estimated between subclades, they should be ranked as four valid pangasiid genera: *Pangasius*, *Pseudolais*, *Helicophagus* and *Pangasianodon*. This conclusion is consistent with the taxonomic study of Thai pangasiids and schilbeids as described by Vidthayanon and Roongthongbaisuree (1993). The previously unclear relationships within the genus *Pangasius* (Pouyaud *et al*., 1998; Pouyaud *et al*., 2000; Pouyaud *et al*., 2004), this molecular study based on combined mitochondrial and nuclear nucleotide sequences recognized three subclades which possibly indicate the existence of three subgenera in the genus *Pangasius* as confirmed by morphological data of Vidthayanon (1993). The newly described species, *Helicophagus leptorhynchus* formed a close relationship with *H. wanndersii*, therefore, they should be considered as conspecific.

The results from this study strongly supported the monophyly of Thai schilbeids including the genera *Laides*, *Clupisoma* and *Eutropiichthys* and the genus *Eutropiichthys* was recognized as Thai basal taxon. The genus *Pseudeutropius* which is native in Sumatra was also included for phylogenetic analysis in this study and it was branched off from Thai schilbeid clade as basal of this family in almost analyses. The enigmatic *Clupisoma sinense* was recovered as more closely related to *Laides longibarbis* than its congeneric species, *C. prateri* which is not congruent with previous taxonomic studies (Vidthayanon and Roongthongbaisuree, 1993; Ng, 1999; Ferraris, 2004; Chen *et al*., 2005). Thus, a recategorization of *C. sinense* to the genus

Laides is suggested and it should be designated as *L. sinensis* as previously proposed by Kottelat (1989), Zakaria-Ismail (1992) and Rainboth (1996). The putatively new species, *Laides longibarbis* and *Eutropiichthys salweenensis* were well-supported as sister to their congeners, *L. hexanema* and *E. vacha*, respectively. Thus, the species status of *L. longibarbis* and *E. salweenensis* was confirmed by this genetic study. This study also confirmed that many genetic loci can give more accurate results and increase the statistical support in phylogenetic studies.

Pangasiidae and Schilbeidae have diverged from a common ancestor probably since Miocene period. The results provided evidence for the divergence within pangasiids which occurred in the late Miocene to the late Pleistocene. The emergence of Schilbeidae initiated at the middle Miocence and extended to the middle Pliocene which was more ancient than those found in Pangasiidae which suggested a long evolution process since the emergence of this family.

Recommendation

Almost all the obtained phylogenies in the present study demonstrated that the intrarelationships within the genus *Pangasius* were largely unresolved. It might be due to restricted number of taxon samplings. Thus, for deep phylogenetic analyses, the broader representatives of the *Pangasius* species distributing beyond the borders of Thailand are needed to improve intrageneric relationships for this genus.

LITERATURE CITED

Almada, F., M. Henriques, A. Levy, A. Pereira, J. Robalo and V. C. Almada. 2008. Reclassification of *Lepodogaster candollei* based on molecular and meristic evidence with a redefinition of the genus *Lepadogaster*. **Mol. Phylogenet. Evol.** 46: 1151-1156.

Alves-Gomes, J. A. 1999. Systematic biology of gymnotiform and mormyriform electric fishes: phylogenetic relationships, molecular clocks and rates of evolution in mitochondrial rRNA genes. **J. Exp. Biol.** 202: 1167–1183.

Apostolidis, A. P., Z. Mamuris and C. Triantaphyllidi. 2001. Phylogenetic relationships among four species of Mullidae (Perciformes) inferred from DNA sequences of mitochondrial *cytochrome b* and *16S rRNA* genes. **Biochem. Syst. Eco.** 29: 901-909.

Avise, J. C. 1994. **Molecular Markers**. Natural History and Evolution, Chapman and Hall, New York.

Bermingham, E. S., S. McCaVerty and A. P. Martin. 1997. Fish biogeography and molecular clocks: Perspectives from the Panamanian Isthmus, pp. 113–128. *In* T. Kocher and C. A. Stepien, eds. **Molecular systematics of fishes.** Academic Press, San Diego.

Bornbusch, A. H. and J. G. Lundberg. 1989. A new species of *Hemisilurus* (Siluriformes, Siluridae) from the Mekong River, with comments on its relationships and historical biogeography. **Copeia.** 1989: 434-444.

Bromham, L. and D. Penny. 2003. The modern molecular clock. **Nat. Rev. Genet.** 4: 216-224.

Brophy, T. R. 2002. **Variation and systematics of the Malayan snail-eating turtle, *Malayemys subtrijuga* (Schlegel and Müller, 1844).** Ph.D. Thesis, George Mason University.

Brown, W. M., E. M. Prager, A. Wang and A. C. Wilson. 1982. Mitochondrial DNA sequences of primates, tempo and mode of evolution. **J. Mol. Evol.** 18: 225-239.

Bufalino, A. P. and R. L. Mayden. 2010. Molecular phylogenetics of North American phoxinins (Actinopterygii: Cypriniformes: Leuciscidae) based on *RAG1* and *S7* nuclear DNA sequence data. **Mol. Phylogenet. Evol.** 55: 274-283.

Burgess, W. E. 1989. **An atlas of freshwater and marine catfishes.** T.F.H. Publishers, New Jersey.

Cabria, M. T., J. Rubines, B. Gómez-Moliner and R. Zardoya. 2006. On the phylogenetic position of a rare Iberian endemic mammal, the Pyrenean desman (*Galemys pyrenaicus*). **Gene.** 375: 1-13.

Castresana, J. 2001. *Cytochrome b* phylogeny and the taxonomy of great apes and mammals. **Mol. Biol. Evol.** 18: 465-471.

Chen, X. Y., C. J. Ferraris and J. X. Yang. 2005. A new species of catfish of the genus *Clupisoma* (Siluriformes: Schilbidae) from the Salween River, Yunnan, China. **Copeia.** 2005: 566-570.

Chen, W. J. and R. L. Mayden. 2009. Molecular systematics of the Cyprinoidea (Teleostei: Cypriniformes), the world'slargest clade of freshwater fishes: Further evidence from six nuclear genes. **Mol. Phylogenet. Evol.** 52: 544-549.

Chiang, T. Y., H. D. Lin, K. T. Shao and K. C. Hsu. 2010. Multiple factors have shaped the phylogeography of Chinese spiny loach *Cobitis sinensis* in Taiwan as inferred from mitochondrial DNA variation. **J. Fish. Biol.** 76: 1173-1189.

Clark, M. K., L. M. Schoenbohm, L. H. Royden, K. X. Whipple, B. C. Burchfiel, X. Zhang, W. Tang, E. Wang and L. Chen. 2004. Surface uplift, tectonic, and erosion of eastern Tibet from large-scale drainage patterns. **Tectonics.** 23: TC1006, doi: 1010.1029/2002TC001402.

Cooper, W. J., L. L. Smith and M. W. Westneat. 2009. Exploring the radiation of a diverse reef fish family: Phylogenetics of the damselfishes (Pomacentridae), with new classifications based on molecular analyses of all genera. **Mol. Phylogenet. Evol.** 52: 1-16.

Crespi, B. J. and M. J. Fulton. 2004. Molecular systematic of Salmonidae: Combined nuclear data yields a robust phylogeny. **Mol. Phylogenet. Evol.** 31: 658-679.

Crow, K. D., Z. Kanamoto and G. Bernardi. 2004. Molecular phylogeny of the hexagrammid fishes using a multi-locus approach. **Mol. Phylogenet. Evol.** 32: 986-997.

Cummings, M. P., S. P. Otto and J. Wakeley. 1995. Sampling properties of DNA sequence data in phylogenetic analysis. **Mol. Biol. Evol**. 12:814-822.

de Pinna, M. C. C. 1998. Phylogenetic relationships of neotropical Siluriformes: history overview and synthesis of hypotheses, pp. 273-330. *In* L. R. Malabarba, R. E. Reis, R. P. Vari, Z. M. Lucena and C. A. S. Lucena, eds. **Phylogeny and classification of neotropical fishes.** EDIPUCR, Porto Alegre.

De Vos, L. 1995. A systematic revision of the African Schilbeidae (Teleostei: Siluriformes). **Ann. Mus. Roy. Afr. Centr.** 271: 1-450.

Diogo, R. 2003. Higher-level phylogeny of Siluriformes - An overview, pp 353-384. *In* B. G. Kapoor, G. Arratia, M. Chardon and R. Diogo, eds. **Catfishes**. Science Publ. Inc., Enfield, USA.

_____, M. Chardon and P. Vandewalle. 2004. Osteology and mycology of the cephalic region and pectoral girdle of *Schilbe mystus*, comparison with other schilbids, and discussion on the monophyly and phylogenetic relationships of the Schilbidae (Teleostei: Siluriformes). **Anim. Biol.** 54: 91-110.

Doadrio, I. and O. Domínguez. 2004. Phylogenetic relationships within the fish family Goodeidae based on *cytochrome b* sequences data. **Mol. Phylogenet. Evol**. 31: 416–430.

_____, S. Perea, L. Alcaraz and N. Hernandez. 2009. Molecular phylogeny and biogeography of the Cuban genus *Girardinus* Poey, 1854 and relationships within the tribe Girardinini (Actinopterygii, Poeciliidae). **Mol. Phylogenet. Evol**. 50: 16-30.

Doiuchi, R. and T. Nakabo. 2006. Molecular phylogeny of the stromateoid fishes (Teleostei: Perciformes) inferred from mitochondrial DNA sequences and compared with morphology-based hypotheses. **Mol. Phylogenet. Evol.** 39: 111-123.

Drummond, A. J. and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. **BMC Evol. Biol.** 7: 214.

Farias, I. P., G. Ortí, I. Sampaio, H. Schneider and A. Meyer. 2001. The *cytochrome b* gene as a phylogenetic marker: The limits of resolution for analyzing relationships among cichlid fishes. **J. Mol. Evol.** 53:89-103.

Farris, J. S., M. Källersjö, A. G. Kluge and C. Bult. 1995. Testing significance of incongruence. **Cladistics**. 10: 315-319.

Felsenstein, J. 1981. Evolutionary tree from DNA sequences: A maximum likelihood approach. **J. Mol. Evol.** 17: 368-376.

Ferraris, C. J., Jr. 2004. A new species of the Asian schilbid catfish genus *Clupisoma* from Myanmar, with a redescription of *Clupisoma prateri* Hora (Osteichthyes: Siluriformes: Schilbidae). **Zootaxa.** 439: 1-10.

_____. 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. **Zootaxa.** 1418: 1-628.

_____ and R. P. Vari. 2007. Revision of catfishes of the genus *Eutropiichthys*, with the description of two new species (Siluriformes: Schilbidae). **Copeia.** 4: 866-885.

Fessler, J. L. and M. W. Westneat. 2007. Molecular phylogenetics of the butterflyfishes (Chaetodontidae): Taxonomy and biogeography of a global coral reef fish family. **Mol. Phylogenet. Evol.** 45: 50-68.

Fitch, W. M., 1971. Toward defining the course of evolution: Minimal change for a specific tree topology. **Syst. Zool**. 20: 406-416.

Geuten, K., E. Smets, P. Schols, Y. M. Yuan, S. Janssens, P. Küpfer and N. Pyck. 2004. Conflicting phylogenies of balsaminoid families and the polytomy in Ericales: Combining data in a Bayesian framework. **Mol. Phylogenet. Evol.** 31: 711-729.

Graybeal, A. 1998. Is it better to add taxa of characters to a difficult phylogenetic problem. **Syst. Biol.** 47: 9-17.

Groth, J. G. and G. F. Barrowclough. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear *RAG1* Gene. **Mol. Phylogenet. Evol.** 12: 115-123.

Guindon, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. **Syst. Biol.** 52: 696-704.

Gustiano, R., G. G. Teugels and L. Pouyaud. 2003. Revision of the *Pangasius kunyit* catfish complex, with description of two new species from South-east Asia (Siluriformes: Pangasiidae). **J. Nat. Hist.** 37: 357-376.

_____, _____ and _____. 2004. *Pangasius bedado* Roberts, 1999: A junior sysonym of *Pangasius djambal* Bleeker, 1846 (Siluriformes, Pangasiidae). **Cybium.** 28: 13-18.

Hall, B. G. 2001. **Phylogenetic trees made easy: A How-to manual for molecular biologists.** Sinauer Associates, Sunderland, MA.

Hardman, M. 2004. The phylogenetic relationships among *Noturus* catfishes (Siluriformes: Ictaluridae) as inferred from mitochondrial gene *cytochrome b* and nuclear *recombination activating gene 2*. **Mol. Phylogenet. Evol.** 30: 395-408.

_____. 2005. The phylogenetic relationships among non-diplomystid catfishes as inferred from mitochondrial *cytochrome b* sequences; the search for the ictalurid sister taxon (Otophysi: Siluriformes). **Mol. Phylogenet. Evol**. 37: 700-720.

_____ and L. M. Page. 2003. Phylogenetic relationships among bullhead catfishes of the genus *Ameiurus* (Siluriformes: Ictaluridae). **Copeia.** 3: 20-33.

Härlid, A., A. Janke and U. Arnason. 1997. The mtDNA sequence of the ostrich and the divergence between paleognathous and neognathous birds. **Mol. Biol. Evol.** 14: 754–761.

Hasegawa, M., H. Kishino and T. Yano. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. **J. of Mol. Evol**. 22: 160-174.

Håstad, O. and M. Björklund. 1998. Nucleotide substitution models and estimation of phylogeny. **Mol. Biol. Evol.** 15: 1381-1389.

He, D. K. and Y. F. Chen. 2007. Molecular phylogeny and biogeography of the highly specialized grade schizothoracine fishes (Teleostei: Cyprinidae) inferred from *cytochrome b* sequences. **Chin. Sci. Bull.** 52: 777-788.

He, S. P., W. X. Cao and Y. Y. Chen. 2001. The uplift of Qinghai-Xizang (Tibet) Plateau and the vicariance speciation of glyptosternoid fishes (Siluriformes: Sisoridae). **Sci. China.** 44: 644-651.

Heath, T. A., S. M. Hedtke and D. M. Hillis. 2008. Taxon sampling and the accuracy of phylogenetic analyses. **J. Syst. Evol.** 46 (3): 239-257.

Heyden, S. V. D. and C. A. Matthee. 2008. Towards resolving familial relationships within the Gadiformes, and the resurrection of the Lyconidae. **Mol. Phylogenet. Evol**. 48: 764-769.

Hillis, D. M. and M. T. Dixon. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. **Q. Rev. Biol.** 66: 411-453.

_____ and J. J. Bull. 1993. An empirical test of booystrapping as a method for assessing confidence in phylogenetic analysis. **Syst. Biol**. 42: 182-192.

Hillis, D. M. and J. J. Wiens. 2000. Molecules versus morphology in systematics: Conflicts, artifacts, and misconceptions, pp. 1–19. *In* J.J. Wiens, eds. **Phylogenetic analysis of morphological data**. Smithsonian Institution Press, Washington DC.

_____, C. Moritz and B. K. Mable. 1996. **Molecular systematic, 2nd edition**. Sinauer Associates, Inc., Sunderland, MA.

Howes, G. J. 1985. The phylogenetic relationships of the electric catfish family Malapteruridae (Teleostei: Siluroidei). **J. Nat. Hist.** 19: 37-67.

Huang, X. and A. Madan. 1999. CAP3: A DNA sequence assembly program. **Genome Res.** 9: 868-877.

Huelsenbeck, J. P., F. R. Ronquist, R. Nielsen and J. P. Bollback. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. **Science.** 294: 2310-2314.

Hulsey, C. D., B. P. Keck and P. R. Hollingsworth, Jr. 2010. Species tree estimation and the historical biogeography of heroine cichlids. **Mol. Phylogenet. Evol.** 58: 124-131.

Hwang, U. W. and W. Kim. 1999. General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. **Korean J. Parasitol.** 37 (4): 215-228.

Inoue, J. C., N. Kumazawa, M. Miya and M. Nishida. 2009. The historical biogeography of the freshwater knifefishes using mitogenomic approaches: A Mesozoic origin of the Asian notopterids (Actinopterygii: Osteoglossomorpha). **Mol. Phylogenet. Evol.** 51: 486-499.

IUCN. 2010. **2010 IUCN red list of threatened species: A global species assessment.** International Union for the Conservation of Nature and Natural Resources. 191 p.

Johns, G. C. and J. C. Avise. 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial *cytochrome b* gene. **Mol. Biol. Evol**. 15: 1481-1490.

Kartavtsev, Y. P., S. O. Jung, Y. M. Lee, H. K. Byeon, and J. S. Lee. 2007. Complete mitochondrial genome of the bullhead torrent catfish, *Liobagrus obesus* (Siluriformes, Amblycipididae): Genome description and phylogenetic considerations inferred from the *cyt b* and *16S rRNA* genes. **Gene.** 396: 13-27.

Kishino, H., J. L. Thorne and W. J. Bruno. 2001. Performance of a divergence time estimation method under a probabilistic model of rate evolution. **Mol. Biol. Evol.** 18: 352-361.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. **Proc. Nat. Acad. Sci.** USA 86: 6196–6200.

_____ and K. L. Carleton. 1997. Base substitution in fish mitochondrial DNA: Patterns and rates, pp. 13-24. *In* T.D. Kocher and C.A. Stepien, eds. **Molecular systematics of fishes.** NY Academic Press, New York.

Kottelat, M. 1989. Zoogeography of the fishes from Indochinese inland waters with an annotated check-list. **Bull. Zool. Mus.** 12: 1-56.

_____. 2001. **Fishes of Laos.** WHT Publications, Colombo, Sri Lanka, 198 p.

Ku, X. Y., Z. G. Peng, R. Diogo and S. P. He. 2007. MtDNA phylogeny provides evidence of generic polyphyleticism for East Asian bagrid catfishes. **Hydrobiologia.** 579: 147-159.

Kumar, S., J. Dudley, M. Nei and K. Tamura. 2008. MEGA: A biologist centric software for evolutionary analysis of DNA and protein sequences. **Brief Bioinform.** 9: 299-306.

Kuo, C. H., S. Huang and S. C. Lee. 2003. Phylogeny of hagfish based on the mitochondrial *16S rRNA* gene. **Mol. Phylogenet. Evol.** 28: 448–457

Li, J., X. Wang, X. Kong, K. Zhao, S. He and R. L. Mayden. 2008. Variation patterns of the mitochondrial *16S rRNA* gene with secondary structure constraints and their application to phylogeny of cyprinine fishes (Teleostei: Cypriniformes). **Mol. Phylogenet. Evol.** 47: 472–487.

Li, C., G. Ortí and J. Zhao. 2010. The phylogenetic placement of sinipercid fishes ("Perciformes") revealed by 11 nuclear loci. **Mol. Phylogenet. Evol.** 56: 1096-1104.

Li, W. H. and D. Graur. 1991. **Fundamentals of molecular evolution.** Sinauer, Sunderland, MA.

Li, X., P. Musikasinthorn and Y. Kumazawa. 2006. Molecular phylogenetic analyses of snakeheads (Perciformes: Channidae) using mitochondrial DNA sequences. **Ichthyol. Res.** 53: 148-159.

Lutzoni, F, P. Wagner, V. Reeb and S. Zoller. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. **Syst. Biol.** 49: 628-651.

Lydeard, C. and K. J. Roe. 1997. The phylogenetic utility of the mitochondrial *cytochrome b* gene for inferring relationships among actinopterygian fishes, pp. *In* T. Kocher and C. Stepian, eds. **Molecular systematics of fishes**. Academic Press, Burlington, MA.

Macey, J. R., J. A. Schulte II, A. Larson, Z. Fang, Y. Wang, B. S. Tuniyev and T. J. Papenfuss. 1998. Phylogenetic relationships of toads in the *Bufo bufo* species group from the eastern escarpment of the Tibetan Plateau: A case of vicariance and dispersal. **Mol. Phylogenet. Evol.** 9: 80–87.

Machordom, A. and I. Doadrio. 2001. Evidence of a Cenozoic Betic–Kabilian connection based on freshwater fish phylogeography (*Luciobarbus*, Cyprinidae). **Mol. Phylogenet. Evol.** 18: 252–263.

Martin, A. P. 1999. Substitution rates of organelle and nuclear genes in sharks: Implicating metabolic rate (again). **Mol. Biol. Evol.** 16: 996-1002.

_____ and E. Bermingham. 1998. Systematics and evolution of lower central American cichlids inferred from analysis of *cytochrome b* gene sequences. **Mol. Phylogenet. Evol.** 9: 192-203.

Meyer, A. 1993. Evolution of mitochondrial DNA in fish, pp. 1-38. *In* P.W. Hochachka and P. Mommsen, eds. **Biochemistry and molecular biology of fish**. Elsevier Press 2.

_____. 1994. DNA Technology and phylogeny of fish, pp. 219-249. *In* A.R. Beaumont, eds. **Genetic and evolution of aquatic organism**. Chapman & Hall, London.

Miller, T. L. and T. H. Cribb. 2007. Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. **Mol. Phylogenet. Evol.** 44: 450-460.

Mo, T., 1991. Anatomy and systematics of Bagridae (Teleostei), and siluroid phylogeny. **Theses Zool.** 17: 1-216.

Moyer, G. R., B. M. Burr and C. Krajewski. 2004. Phylogenetic relationships of thorny catfishes (Siluriformes: Doradidae) inferred from molecular and morphological data. **Zool. J. Linn. Soc.** 140: 551-575.

Nei, M. and S. Kumar. 2000. **Molecular evolution and phylogenetics**. Oxford University Press, New York.

Ng, H. H. 1999. *Laides longibarbis*, a valid species of schilbeid catfish from Indochina (Teleostei: Siluriformes). **Ichthyol. Explor. Freshwaters.** 10: 381-385.

_____. 2002. *Bagrichthys majusculus*, a new catfish from Indochina (Teleostei, Bagridae). **Folia Zool.** 51: 49-54.

_____. 2003. *Kryptopterus geminus*, a new species of silurid catfish (Teleostei: Siluridae) from mainland Southeast Asia. **Zootaxa.** 305: 1-11.

_____. 2004. *Wallago micropogon*: A new species of silurid catfish (Teleostei: Siluridae) from mainland Southeast Asia. **Copeia.** 1: 92-97.

_____ and J. Freyhof. 2005. A new species of *Pseudomystus* (Teleostei: Bagridae) from central Vietnam. **Copeia.** 4: 745-750.

Ng, H. H. and M. Kottelat. 2000. *Helicophagus leptorhynchus*, a new species of molluscivorous catfish from Indochina (Teleostei: Pangasiidae). **Raff. Bull. Zool.** 48: 55-58.

Nelson, J. S. 1976. **Fishes of the world**. A Wiley-Interscience Publ. John Wiley and Sons, Inc., New York.

_____. 1994. **Fishes of the world**. A Wiley-Interscience Publ. John Wiley and Sons, Inc., New York.

Orrell, T. M. 2000. **A molecular phylogeny of the Sparidae (Perciformes: Percoidei).** Ph.D. Thesis, The College of William and Mary in Virginia.

_____ and K. E. Carpenter. 2004. A phylogeny of the fish family Sparidae (porgies) inferred from mitochondrial sequence data. **Mol. Phylogenet. Evol.** 32: 425-434.

Ortí, G., P. Petry, J. I. R. Porto, M. Jegu, and A. Meyer. 1996. Patterns of nucleotide change in mitochondrial ribosomal RNA genes and the phylogeny of piranhas. **J. Mol. Evol.** 42: 169-182.

Page, R. D. M. and E. C. Holmes. 1998. **Molecular evolution: A phylogenetic approach**. Blackwell Science Ltd., Oxford, UK.

Palumbi, S., A. Martin, S. Romano, W. McMillan, L. Stice and G. Grabowski. 1991. **The simple fools guide to PCR.** Univ. of Hawaii Press, Honolulu, HI.

Peng, Z. G., S. P. He and Y. G. Zhang. 2002. Mitochondrial *cytochrome b* sequence variations and phylogeography of the East Asian bagrid catfishes. **Prog. Nat. Sci.** 12: 421–425.

Peng, Z. G., S. P. He and Y. G. Zhang. 2004. Phylogenetic relationships of glyptosternoid fishes (Siluriformes: Sisoridae) inferred from mitochondrial *cytochrome b* gene sequences. **Mol. Phylogenet. Evol.** 31: 979-987.

_____, S. Y. W. Ho, Y. G. Zhang and S. P. He. 2006. Uplift of the Tibetan plateau: Evidence from divergence times of glyptosternoid catfishes. **Mol. Phylogenet. Evol.** 39: 568-572.

Perdices, A., J. Bohlen and I. Doadrio. 2008. The molecular diversity of Adriatic spined loaches (Teleostei, Cobitidae). **Mol. Phylogenet. Evol.** 46: 382-390.

_____ and I. Doadrio. 2001. The molecular systematics and biogeography of the European cobitids based on mitochondrial DNA sequences. **Mol. Phylogenet. Evol.** 19: 468–478.

_____, _____ and E. Bermingham. 2005. Evolutionary history of the synbranchid eels (Teleostei: Synbranchidae) in Central America and the Caribbean islands inferred from their molecular phylogeny. **Mol. Phylogenet. Evol.** 37: 460-473.

_____, _____, P. S. Economidis and J. Bohlen. 2003. Pleistocene effects on the European freshwater fish fauna: Double origin of the cobitid genus *Sabanejewia* in the Danube basin (Osteichthyes: Cobitidae) **Mol. Phylogenet. Evol.** 26: 289–299.

Pesole, G., C. Gissi, A. D. Chirico and C. Saccone. 1999. Nucleotide substitution rate of mammalian mitochondrial genomes. **J. Mol. Evol.** 48: 427–434.

Philasamorn, C. and R. Satrawaha. 2009. Redescription of *Bagrichthys macropterus* and comparing it with *B. macracanthus*, *B. majusculus* and *B. obscurus*. **Turk. J. Fish. Aquat. Sci.** 9: 9-15.

Pollock, D. D., D. J. Zwickl, J. A. Mcguire and D. M. Hillis. 2002. Increased taxon sampling is advantageous for phylogenetic inference. **Syst. Biol.** 51: 664-671.

Posada, D. and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. **Bioinformatics.** 14: 817-818.

Pouyaud, L., R. Gustiano and M. Legendre. 1998. Phylogenetic relationships among pangasiid catfish species (Siluriformes, Pangasiidae) and new insights on their zoogeography, pp. 49-56. *In* **Proceedings of the mid-term workshop of the catfish Asia project**. 11-15 May 1998, Can Tho, Vietnam.

_____, R. Gustiano and G. G. Teugels. 2002. Systematic revision of *Pangasius polyuranodon* (Siluriformes, Pangasiidae) with description of two new species. **Cybium.** 26: 243-252.

_____, _____ and _____. 2004. Contribution to the phylogeny of the Pangasiidae based on mitochondrial *12S rDNA*. **Indones. J. Agri. Sci.** 5: 45-62.

_____ and G. G. Teugels. 2000. Description of a new pangasiid catfish from East Kalimantan, Indonesia (Siluriformes: Pangasiidae). **Ichthyol. Explor. Freshwaters.** 7: 185-191.

_____, _____, R. Gustiano and M. Legendre. 2000. Contribution to the phylogeny of pangasiid catfishes based on allozymes and mitochondrial DNA. **J. Fish. Biol.** 56: 1509-1538.

_____, _____ and M. Legendre. 1999. Description of a new pangasiid catfish from South-East Asia (Siluriformes). **Cybium.** 23: 247-258.

Quenouille, B., E. Bermingham, and S. Planes. 2004. Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA seqyences. **Mol. Phylogenet. Evol.** 31: 66-88.

Rainboth, W. J. 1991. Cyprinids of Southeast Asia, pp. 156-210. *In* I. J. Winfield and J. S. Nelson, eds. **Cyprinid fishes: Systematics, biology and exploitation.** Chapman and Hall, London.

_____. 1996. **Fishes of the Cambodian Mekong.** FAO identification sheets for fishery 367 purposes. Food and Agriculture Organization, Rome.

Raselimanana, A. P., B. Noonan, K. P. Karanth, J. Gauthier and A. D. Yoder. 2009. Phylogeny and evolution of Malagasy plated lizards. **Mol. Phylogenet. Evol.** 50: 336-344.

Reed, D. L., M. J. deGravelle and K. E. Carpenter. 2001. Molecular systematic of *Selene* (Perciformes: Carangidae) based on *cytochrome b* sequences. **Mol. Phylogenet. Evol.** 21: 468-475.

Ritchie, P. A., L. Bargelloni, A. Meyer, J. A. Taylor, J. A. Macdonald and D. M. Lambert. 1996. Mitochondrial phylogeny of trematomid fishes (Nototheniidae, Perciformes) and the evolution of Antarctic fish. **Mol. Phylogenet. Evol.** 5: 383-390.

Robert, T. R. 1989. The freshwater fishes of Western Borneo (Kalimantan Barat, Indonesia). **Mem. California Acad. Sci.** 14: 1-210.

_____ and J. Jumnongthai. 1999. Miocene fishes from Lake Phetchabun in North-central Thailand, with descriptions of new taxa of Cyprinidae, Pangasiidae and Chandidae. **Nat. Hist. Bull. Siam Soc.** 47: 153-189.

Robert, T. R. and C. Vidthayanon. 1991. Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. **Proc. Acad. Nat. Sci. Philadelphia.** 143: 97-144.

Rocha, L. A., K. C. Lindeman, C. R. Rocha and H. A. Lessios. 2008. Historical biogeography and speciation in the reef fish genus *Haemulon* (Teleostei: Haemulidae). **Mol. Phylogenet. Evol.** 48: 918-928.

Rodríguez, F., J. F. Oliver, A. Marín and J. R. Medina. 1990. The general stochastic model of nucleotide substitution. **J. Theor. Biol**. 142: 484-501.

Ronquist, F. R. and J. P. Huelsenbeck. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. **Bioinformatics** 19: 1572-1574.

Rüber, L., R. Britz, S. O. Kullander and R. Zardoya. 2004. Evolutionary and biogeographic patterns of the Badidae (Teleostei: Perciformes) inferred from mitochondrial and nuclear DNA sequence data. **Mol. Phylogenet. Evol.** 32: 1010-1022.

_____, _____ and R. Zardoya. 2006. Molecular phylogenetic and evolutionary diversification of labyrinth fishes (Perciformes: Anabantoidei). **Syst. Biol.** 55: 374-397.

_____, J. L. Van Tassell and R. Zardoya. 2003. Rapid speciation and ecological divergence in the American seven-spined gobies (Gobiidae, Gobiosomatini) inferred from a molecular phylogeny. **Evolution** 57: 1584-1598.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. **Mol. Biol. Evol.** 4: 406-425.

Salemi, M. and A. M. Vandamme. 2003. **The phylogenetic handbook: A practical approach to DNA and protein phylogeny.** Cambridge university press, Cambridge.

San Mauro, D., D. J. Gower, O. V. Oommen, M. Wilkinson and R. Zardoya. 2004. Phylogeny of caecilian amphibians (Gymnophiona) based on complete mitochondrial genomes and nuclear *RAG1*. **Mol. Phylogenet. Evol.** 33: 413-427.

_____. 2006. **Molecular evolution and phylogenetics of caecilian amphibians (Gymnophiona).** Ph.D. Thesis, Universidad Autonoma de Madrid.

Schatz, D. G. 2004. Antigen receptor genes and the evolution of recombinase. **Sem. Immunol.** 16: 245-256.

Schönhuth, S., I. Doadrio, O. Domingguez, D. M. Hillis and R. L. Mayden. 2008. Molecular evolution of southern North American Cyprinidae (Actinopterygii), with the description of the new genus *Tampichthys* from central Mexico. **Mol. Phylogenet. Evol.** 47: 729-756.

Shimabukuro-Dias, C. K., C. Oliveira, R. E. Reis and F. Foresti. 2004. Molecular phylogeny of the armored catfish family Callichthyidae (Ostariophysi, Siluriformes). **Mol. Phylogenet. Evol.** 32: 152-163.

Šlechtová, V., J. Bohlen, J. Freyhof and P. Ráb. 2006. Molecular phylogeny of the Southeast Asian freshwater fish family Botiidae (Teleostei: Cobitoidea) and the origin of polyploidy in their evolution. **Mol. Phylogenet. Evol.** 39: 529-541.

Šlechtová, V., J. Bohlen and A. Perdices. 2008. Molecular phylogeny of the freshwater fish family Cobitidae (Cypriniformes: Teleostei): Delimitation of genera, mitochondrial introgression and evolution of sexual dimorphism. **Mol. Phylogenet. Evol.** 32: 545-562.

Sloss, B. L., N. Billington and B. M. Burr. 2004. A molecular phylogeny of the Percidae (Teleostei, Perciformes) based on mitochondrial DNA sequence. **Mol. Phylogenet. Evol.** 32: 545-562.

Smith, H. M., 1945. **The freshwater fishes of Siam or Thailand.** Bull. U. S. Nat. Mus. 188, xxii+163 pp.

Song, C. B., T. J. Near and L. M. Page. 1998. Phylogenetic relations among percid fishes as inferred from mitochondrial *cytochrome b* DNA sequence data. **Mol. Phylogenet. Evol.** 10: 343-353.

Strimmer, K. and A. von Haeseler. 2003. Nucleotide substitution models, pp. 72-100. *In* M. Salemi and A. M. Vandamme, eds. **The phylogenetic handbook: A practical approach to DNA and protein phylogeny.** Cambridge University Press, Cambridge.

Sullivan, J. P., J. G. Lundberg and M. Hardman. 2006. A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using *rag1* and *rag2* nuclear gene sequences. **Mol. Phylogenet. Evol.** 41: 636-662.

Swofford, D. L. 2002. **PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b 10.** Sinauer Associates, Inc., Sunderland, MA, US.

Taki, Y. 1978. Geographic distribution of primary freshwater fishes in four principal areas of Southeast Asia. **Tonan Ajia Kenkyukyu (Southeast Asia Studies).** 13: 200-214.

Talwar, P. K. and A. G. Jhingran. 1991. **Inland fishes of India and adjacent countries.** Oxford & IBH Publishing Co, New Delhi, Bombay, Calcutta.

Teugels, G. G. 1996. Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi, Siluroidei): An overview. **Aquat. Living Resour.** 9: 9-34.

_____. 2003. State of the art of recent siluriform systematic, pp. 317-352. *In* B. G. Kapoor, G. Arratia, M. Chardon and R. Diogo, eds. **Catfishes.** Science Publishers Inc., Enfield, USA.

Thorne, J. L. and H. Kishino. 2002. Divergence time and evolutionary rate estimation with multilocus data. **Syst. Biol.** 51: 689-702.

_____, _____ and I. S. Painter. 1998. Estimating the rate of molecular evolution. **Mol. Biol. Evol.** 15: 1647-1657.

Vences, M., J. Freyhof, R. Sonnenberg, J. Kosuch and M. Veith. 2001. Reconciling fossils and molecules: Cenozoic divergence of cichlid fishes and the biogeography of Madagascar. **J. Biogeogr.** 28: 1091-1099.

Vidthayanon, C. 1993. **Taxonomic revision of the catfish family Pangasiidae.** Ph.D. Thesis, Tokyo University.

_____ and S. Roongthongbaisuree. 1993. **Taxonomy of Thai riverine catfishes family Schilbeidae and Pangasiidae.** NIFI Tech. Pap. 150, 58p.

Voris, H. K. 2000. Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. **J. Biogeogr.** 27: 1153-1167.

Waldbieser,G. C., A. L. Bilodeau and D. J. Nonneman. 2003. Complete sequence and characterization of the channel catfish mitochondrial genome. **DNA Seq.** 14: 265-277.

Wang, H. Y. and S. C. Lee. 2002. Secondary structure of mitochondrial *12S rRNA* among fish and its phylogenetic applications. **Mol. Biol. Evol.** 19: 138-148.

Watanabe, K., N. H. Jang-Liaw, C. G. Zhang, S. R. Jeon and M. Nishida. 2007. Comparative phylogeography of bagrid catfishes in Taiwan. **Ichthyol. Res.** 54: 253-261.

Wiley, E. O., G. D. Johnson and W. W. Dimmick. 1998. The phylogenetic relationships of Lampridiform fishes (Teleosteoi: Acanthomorpha), based on a total-evidence analysis of morphological and molecular data. **Mol. Phylogenet. Evol.** 10: 417-425.

Zakaria-Ismail, M. 1992. Notes on the catfish *Laides sinensis* (Pisces: Pangasiidae) from Peninsular Malaysia. **Malay. Nat. J.** 46: 111-114.

Zardoya, R. and I. Doadrio. 1999. Molecular evidence on the evolutionary and biogeographical patterns of European cyprinids. **J. Mol. Evol.** 49: 227–237.

_____ and A. Meyer. 1996. Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates. **Mol. Biol. Evol**. 13: 933-942.

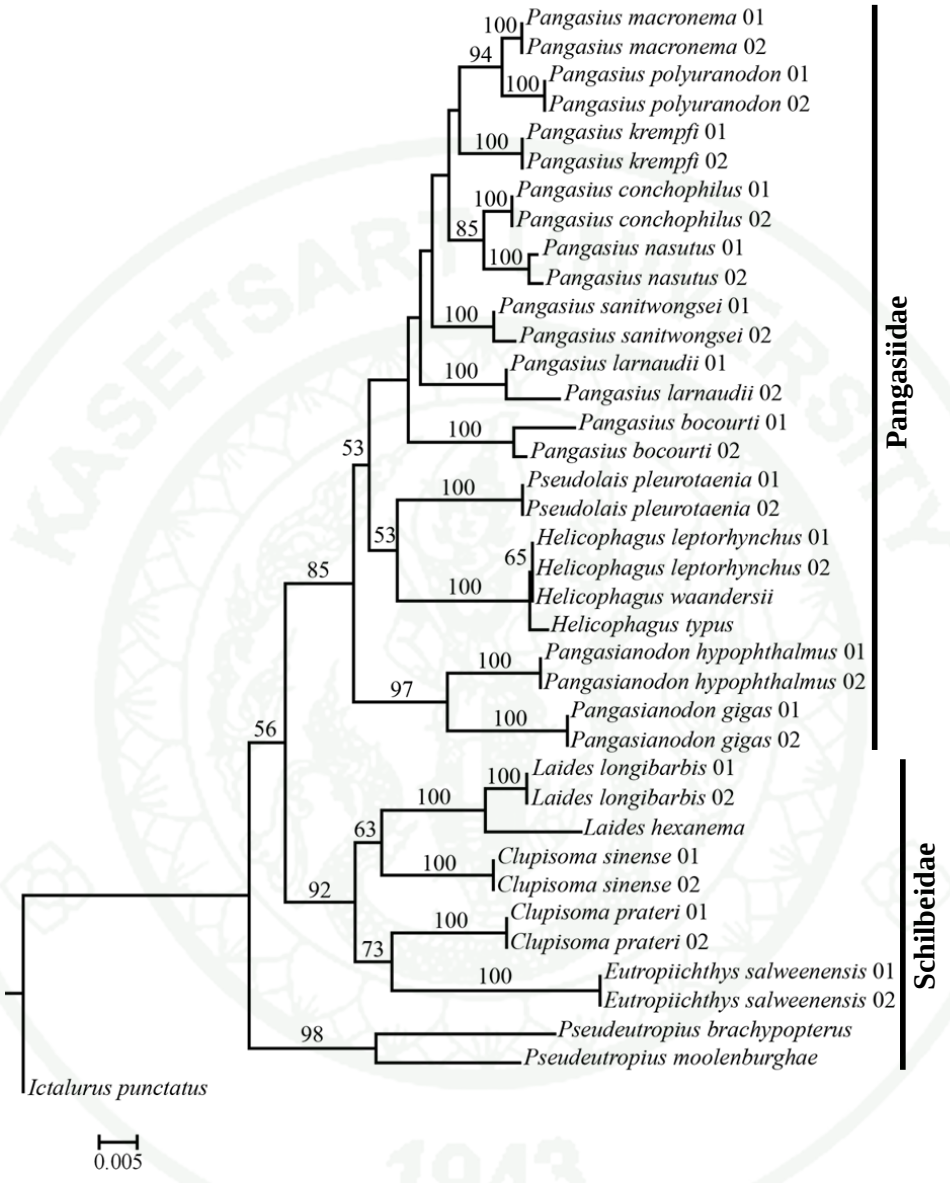
_____ and _____. 2000. Mitochondrial evidence on the phylogenetic position of Caecilians (Amphibia: Gymnophiona). **Genetics.** 155: 765-775.

Zharkikh, A. and W. H. Li. 1992. Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences: I. Four taxa with a molecular clock. **Mol. Biol. Evol.** 9: 1119–1147.

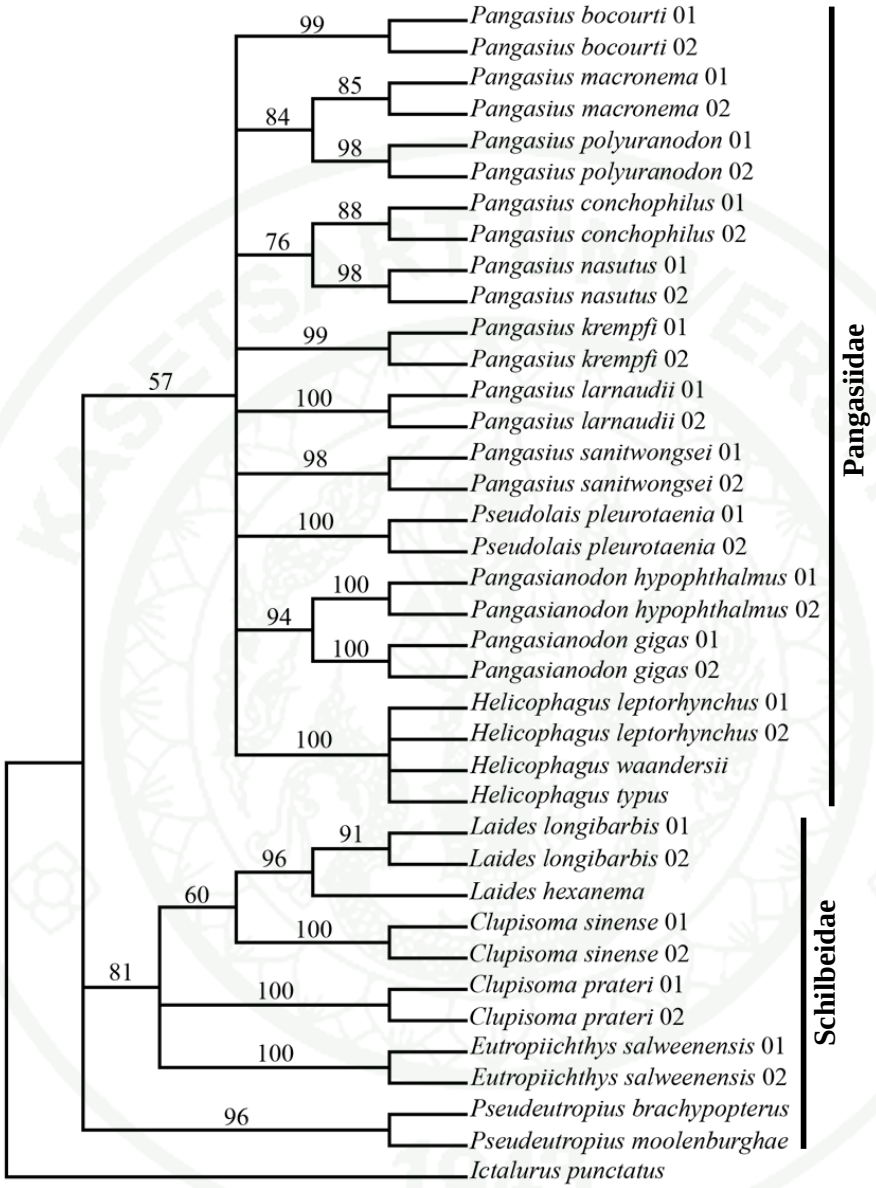
Zuckerkandl, E. and L. Pauling. 1965. Evolutionary divergence and convergence in proteins, pp. 97-166. *In* V. Bryson and H. Vogel, eds. **Evolving genes and proteins.** Academic Press, New York.

Zwickl, D. J. and D. M. Hillis. 2002. Increased taxon sampling greatly reduces phylogenetic error. **Syst. Biol.** 51: 588-598.

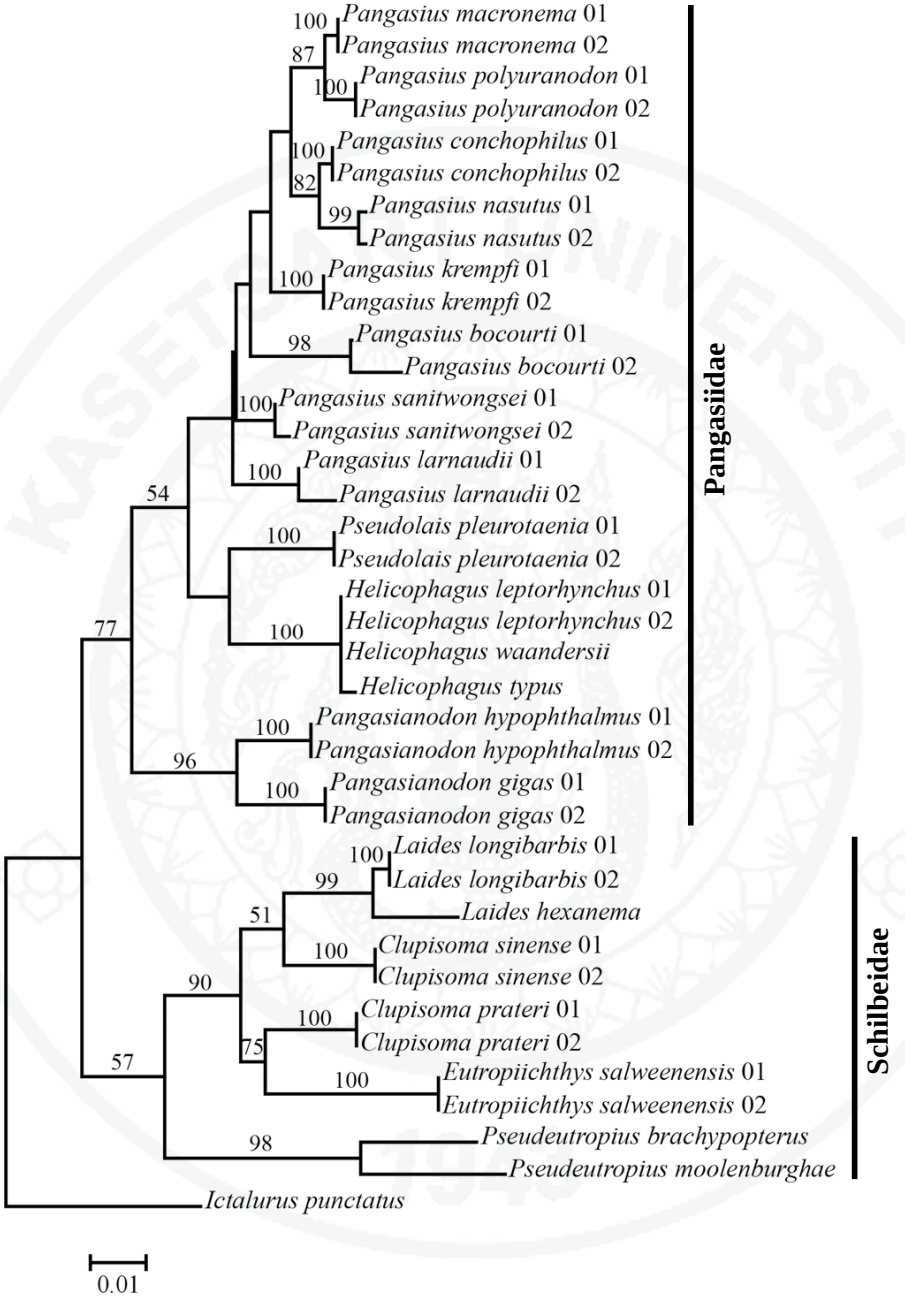
APPENDIX



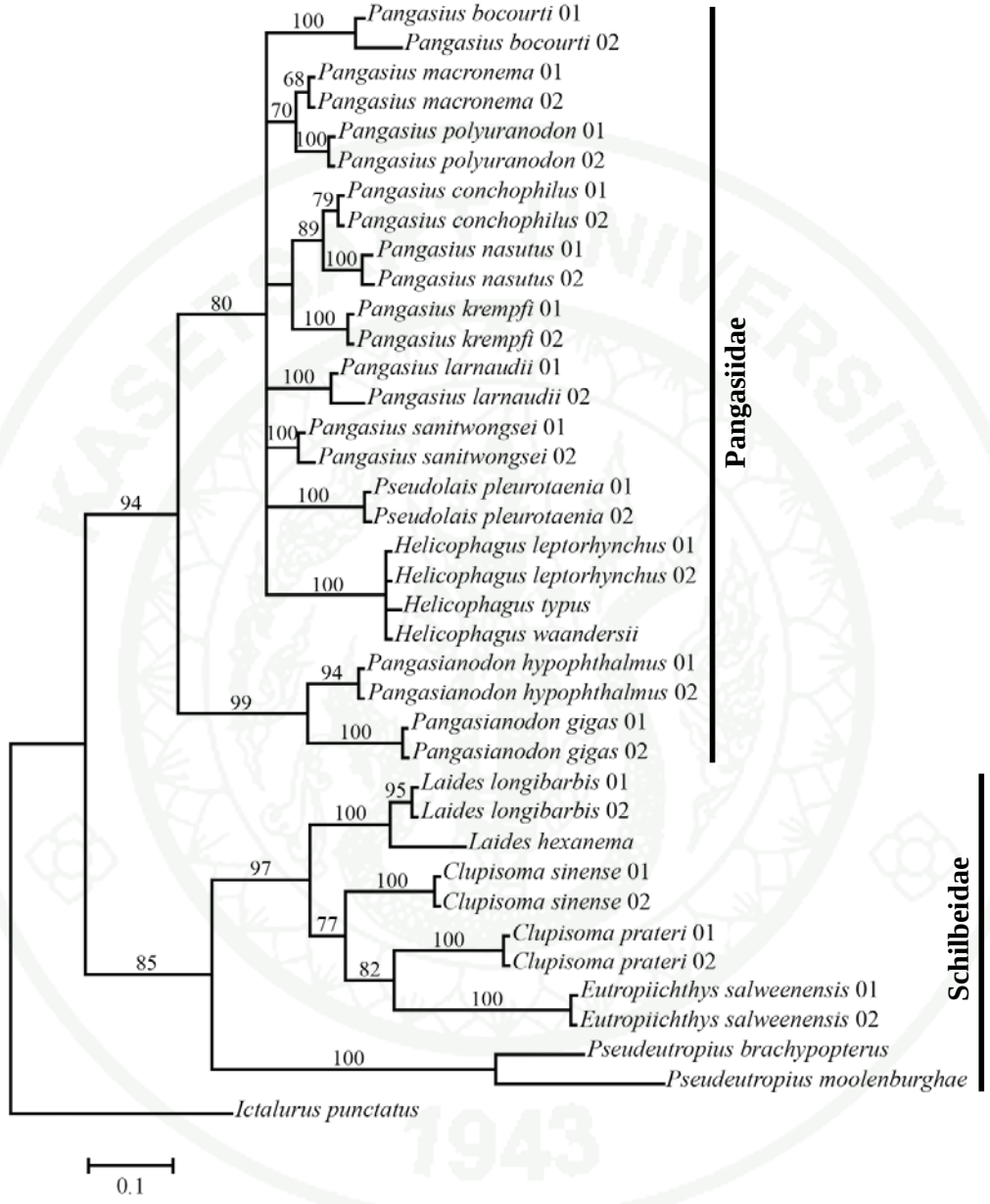
Appendix Figure 1 NJ tree of Thai pangasiids and schilbeids inferred from *cyt b* sequences without nucleotides at third codon positions. Numbers above branches represent bootstrap support for NJ (1,000 replicates). Nodes with support value below 50% are not numbered.



Appendix Figure 2 MP tree of Thai pangasiids and schilbeids inferred from *cyt b* sequences without nucleotides at third codon positions. Numbers above branches represent bootstrap support for MP (1,000 replicates). Nodes with support value below 50% are not numbered.



Appendix Figure 3 ML tree of Thai pangasiids and schilbeids inferred from *cyt b* sequences without nucleotides at third codon positions. Numbers above branches represent bootstrap support for ML (500 replicates). Nodes with support value below 50% are not numbered.



Appendix Figure 4 BI tree of Thai pangasiids and schilbeids inferred from *cyt b* sequences without nucleotides at third codon positions. Numbers above branches represent the posterior probability percentage for BI. Nodes with support value below 50% are not numbered.

CURRICULUM VITAE

NAME : Ms. Wirangrong Karinthanyakit

BIRTH DATE : April 2, 1981

BIRTH PLACE : Nakhonsawan, Thailand

EDUCATION :

YEAR	INSTITUTE	DEGREE/DIPLOMA
2003	Kasetsart Univ.	B.Ed. (Biology)

CONFERENCE : Poster presentation in the 9th RGJ-Ph.D. Congress, Pattaya, Cholburi, Thailand.
"Molecular phylogenetic relationships among Pangasiidae and Schilbeidae in Thailand". 4-6 April 2008.