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PRIMARY RESEARCH PAPER

MtDNA phylogeny provides evidence of generic polyphyleticism for East Asian bagrid catfishes

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Abstract We explore the intrafamilial relationships of East Asian bagrid catfishes (*Hemibagrus*, *Pseudobagrus*, *Pelteobagrus*, and *Leiocassis*) based on 245 sequences of 1092 bp mitochondrial cytochrome *b* fragments. Four haplotypes were found to be shared by *Pseudobagrus ussuriensis*, *Pelteobagrus vachelli* and *Pelteobagrus nitidus*. Phylogenetic trees were performed using the neighbor joining, maximum parsimony, maximum likelihood, and Bayesian likelihood methods. The phylogenetic trees based on NJ, MP, ML and BL inferences strongly support polyphyleticism for the currently recognized genera *Pseudobagrus*, *Pelteobagrus* and *Leiocassis*. However, the species currently assigned to these three genera form a robustly monophyletic group with relatively low genetic divergence. The structure of maxillary barbels and serrations on the anterior edge of the pectoral spines seem to be indicatory of appropriate phylogenetic traits. We propose that only *Hemibagrus* and *Pseudobagrus* are the only valid genera of East Asian bagrids.

Keywords Bagridae · Siluriformes · Molecular phylogeny · Cytochrome *b* · Taxonomy

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Introduction

Historically, attempts to elucidate evolutionary relationships among members of family Bagridae (Teleostei: Siluriformes) were not as numerous as for other catfishes. It may be largely the consequence of the overwhelming species diversity and their wide distribution (e.g., Mo, 1991; Ng, 2003). The bagrid catfishes occur widely in both fresh and brackish water throughout Asia (Southeast, South and East Asia) and Africa. It is a large and morphologically diverse group comprising up at least 144 species belonging to 18 genera, according to a recent overview by Teugels (2003).

The Bagridae *sensu* Mo (1991) is generally accepted by most catfish specialists though there is still some uncertainty regarding the monophyly or the validity of some genera (e.g., Diogo et al., 1999; Diogo, 2003, 2004; Ng, 2003; Teugels, 2003;

Table 1 Different hypotheses concerning the composition of family Bagridae

	Subfamily	Classifications of genera
Regan (1911)	Chrysichthyinae	*Pseudobagrus*, *Gephyroglanis*, *Clarotes*, *Chrysichthys, Rita*; *Auchenoglanis*, *Notoglanidium*, *Parauchenoglanis*
	Bagrinae	*Bagrus*, *Mystus*, *Leiocassis*, *Bagroides*, *Olyra*
	Chrysichthyinae	Pelteobagrini: *Pseudobagrus*, *Pelteobagrus*, *Coreobagrus*, *Horabagrus* Chrysichthyini: *Chrysichthys*, *Amarginops*, *Clarotes*, *Gnathobagrus* Gephyroglanidini: *Gephyroglanis*, *Leptoglanis*, *Phyllonemus*
Jayaram (1968)	Bagrinae	*Porcus* (renamed *Bagrus* later), *Mystus*, *Leiocassis*, *Heterobagrus*
	Ritinae	*Rita*, *Rama*
	Auchenoglanidinae	*Auchenoglanis*, *Notoglanidium*, *Parauchenoglanis*, *Liauchenoglanis*
	Bagroidinae	*Bagroides*, *Bagrichthys*
Mo (1991)	Bagrinae	*Aorichthys*, *Bagrichthys*, *Bagroides*, *Batasio*, *Pseudomystus*, *Olyra*, *Neotropius*, *Mystus*, *Pseudobagrus*, *Leiocassis*, *Pelteobagrus*, *Hemibagrus*, *Bagrus*
	Ritinae	*Rita*, *Nanobagrus*
Ng (2003)		*Bagrichthys*, *Bagroides*, *Bagrus*, *Batasio*, *Pseudomystus*, *Olyra*, *Neotropius*, *Mystus*, *Pseudomystus*, *Rama*, *Rita*, *Sperata*, *Hyalobagrus*, *Hemileiocassis*, *Hemibagrus* *Pseudobagrus*, *Leiocassis*, *Pelteobagrus*

see also Table 1). According to Mo (1991), the bagrid genera *Pseudobagrus* plus *Pelteobagrus* are native to East Asia except for a questionable *Pelteobagrus ornatus* in Malaysia. In total, there are about 40 bagrid species of four different genera (*Pseudobagrus*, *Pelteobagrus*, *Leiocassis*, and *Hemibagrus*) occurring in East Asia, i.e. China, Japan, Korean Peninsula, eastern Siberia and northern Vietnam.

Despite previously considerable morphological, allozyme and osteological work (e.g., Tilak, 1965; Mo, 1991; Maeda et al., 1994; Dai et al., 1998; Diogo et al., 1999), the phylogenetic relationship within the Bagridae are still equivocal. Mo (1991) undertook a comprehensive morphological work on this group but his results failed to fully resolve the generic relations, especially those among Chinese species. Okazaki et al. (1999) sequenced cytochrome *b* (cyt *b*) fragments to investigate the phylogeny of the bagrid catfishes. They stressed that, in order to solve the general phylogeny of this family, it is crucial to solve the intrarelationships of Chinese bagrids since the bagrid groups that display a greater number of species are precisely those distributed in Mainland China. Peng et al. (2002) studied the phylogeny of East Asian bagrids using cyt *b* sequences, but their study resulted in relatively low phylogenetic information, as the Chinese samples included were rather incomplete in their taxon coverage.

The starting point of our work is to obtain a better understanding of the intrafamilial relationships among East Asian bagrids, by uniting previous limited molecular information with a comprehensive sampling of Chinese bagrid populations in the present study.

Materials and methods

Sample collection

Here we adopt the classification of the Bagridae *sensu* Chu et al. (1999). Since we detected in preliminary studies by accident that different species shared the same haplotypes, taxon sampling was increased to a total of 245 sequences in order to reduce phylogenetic error (Zwickl & Hillis, 2002). For the 245 sequences, 242 represent 23 bagrid species and the remaining 3 represent outgroups. Among the 245 sequences, 27 were retrieved from GenBank and 218 were newly determined. All the specimens were deposited in the Fish Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. All tissues used for DNA extraction were preserved in 95% ethanol and all the sequences have been deposited in GenBank. Collection localities and species vouchers are given in Table 2. Due to the ambiguity concerning the identity of the sister-group of bagrids (e.g., Diogo, 2003), members of

Table 2 Bagrid samples used in the present study and their locations

Taxon and localities	Haplotype	Size	Species-voucher[a]	AC No.
Pelteobagrus fulvidraco				
Yueyang, Hunan	F1	1	IHB 0305154	AY912282
	F3	1	IHB 0305155	AY912288
	F5	1	IHB 0305156	AY912291
	F6	2	IHB 0305153, 0305157	AY912298
Taoyuan, Hunan	F6	1	IHB 0307248	AY912297
	F7	1	IHB 0307249	AY912301
Jinkou, Wuhan	F2	1	IHB 0380607	AY912283
	F3	1	IHB 0305180	AY912285
	F6	3	IHB 0380608–0380610	AY912293
Jiangxia, Wuhan	F3	2	IHB 0380612, 0380316	AY912284
	F6	3	IHB 0380611, 0380617–0380618	AY912292
Duchang, Jiangxi	F3	2	IHB 0305185–0305186	AY912286
	F4	1	IHB 0305182	AY912290
	F6	2	IHB 0305183–0305184	AY912294
Shangrao, Jiangxi	F3	1	IHB 0305193	AY912287
	F6	7	IHB 0305187–0305192, 0305194	AY912295
	F6	1		AF430376*
Leshan, Sichuan	F3	1	IHB 0305196	AY912289
Hechuan, Chongqing	F6	1	IHB 0405212	AY912296
	F19	1	IHB 0405213	AY912316
Jian'ou, Fujian	F6	1	IHB 0404208	AY912299
	F13	2	IHB 0404209–0404210	AY912311
Jianyang, Fujian	F6	1	IHB 0305204	AY912300
Changting, Fujian	F20	1	IHB 0404214	AY912317
	F21	1	IHB 0404213	AY912318
Yangshuo, Guangxi	F8	3	IHB 0305161, 0305163, 0404215	AY912302
	F9	1		AF430375*
	F10	1	IHB 0305162	AY912305
	F11	1	IHB 0404216	AY912308
	F12	1	IHB 0404217	AY912310
Jinxiu, Guangxi	F8	1	IHB 0305164	AY912303
Yongfu, Guangxi	F8	2	IHB 0404218–0404219	AY912304
	F11	1	IHB 0404220	AY912309
Rong'an, Guangxi	F10	1	IHB 0305195	AY912306
Liuzhou, Guangxi	F10	1	IHB 0305206	AY912307
Biliuhe, Liaoning	F14	1	IHB 0305166	AY912312
	F15	1	IHB 0305175	AY912313
Kaiyuan, Liaoning	F16	1	IHB 0307242	AY912314
	F17	1	IHB 0305205	AY912315
Tonglu, Zhejiang	F22	1	IHB 0404211	AY912319
	F23	1	IHB 0404212	AY912320
Korea	F18	1		AB015992*
Pelteobagrus eupogon				
Jinkou, Wuhan	E1	1	IHB 0305179	AY912323
	E4	1	IHB 0305176	AY912326
	E6	1	IHB 0305181	AY912329
	E9	1	IHB 0305177	AY912332
	E10	1	IHB 0305178	AY912333
Yueyang, Hunan	E2	1	IHB 0305202	AY912324
	E3	2	IHB 0305198–0305199	AY912325
	E4	3	IHB 0305197, 0305200–0305201	AY912327
	E5	1	IHB 0305158	AY912328
	E7	1	IHB 0305203	AY912330
	E8	1	IHB 0305159	AY912331
Pelteobagrus nitidus				

Table 2 continued

Taxon and localities	Haplotype	Size	Species-voucher[a]	AC No.
Ha'erbin	N1	2	IHB 0305233, 0305238	AY912334
	N3	1	IHB 0305234	AY912335
	N4	4	IHB 0305235–0305237, 0305239	AY912336
Fuyang, Zhejiang	N6	1	IHB 0305218	AY912337
	N7	1	IHB 0305220	AY912338
	N10	1	IHB 0305217	AY912341
	N11	1		AF416893*
	N15	1	IHB 0305216	AY912345
	N17	1	IHB 0305215	AY912347
	N22	1	IHB 0305219	AY912353
Jian'ou, Fujian	N19	1	IHB 0404202	AY912349
	N20	1	IHB 0404205	AY912350
	N21	1	IHB 0404203	AY912351
	N22	1	IHB 0404204	AY912354
Jianyang, Sichuan	N18	1	IHB 0305246	AY912348
Yueyang, Hunan	N8	1	IHB 0305214	AY912339
	N14	1	IHB 0305211	AY912344
	N16	1	IHB 0305210	AY912346
	N22	1	IHB 0305212	AY912352
Taoyuan, Hunan	N9	1	IHB 0307258	AY912340
	N12	1	IHB 0307256	AY912342
	N13	1	IHB 0307257	AY912343
Hejiang, Sichuan	U3	1	IHB 0404297	AY912355
	U6	2	IHB 0404298–0404299	AY912356
	U8	1	IHB 0404296	AY912357
Korea	N2	1		AB015994*
Russia	N5	1		AB015993*
Pelteobagrus vachelli				
Hechuan, Chongqing	V1	1	IHB 0405214	AY912358
	V5	2	IHB 0405215, 0405217	AY912362
	V12	1	IHB 0405216	AY912369
Zhangping, Fujian	V3	1	IHB 0404206	AY912360
	V4	1	IHB 0404207	AY912361
Fujian, Jian'ou	V6	1		AF416896*
Yueyang, Huana	V2	1	IHB 0305213	AY912359
Chongzuo, Guangxi	V7	1	IHB 0380507	AY912363
Tengxian, Guangxi	V8	1	IHB 0404290	AY912364
	V9	3	IHB 0404291–0404292, 0404294	AY912365
	V10	1	IHB 0404293	AY912366
Hejiang, Sichuan	V11	1	IHB 0404223	AY912368
	U3	1	IHB 0404302	AY912370
	U8	2	IHB 0404301, 0404305	AY912371
	U10	1	IHB 0404304	AY912372
Pseudobagrus ondon				
Changting, Fujian	O1	3	IHB 0404198–0404200	AY912373
	O2	1	IHB 0404196	AY912374
	O4	1	IHB 0404197	AY912376
Jian'ou, Fujian	O3	1	IHB 0404194	AY912375
Fuyang, Zhejiang	O5	1	IHB 001101004	AY912377
Pseudobagrus tenuis				
Tonglu, Zhejiang	TE1	3	IHB 0404190–0404192	AY912381
	TE2	1	IHB 0404193	AY912382
	TE3	1	IHB 0404189	AY912383
Pseudobagrus ussuriensis				
Fuyang, Zhejiang	U1	1	IHB 001101007	AY912392
	U10	1	IHB 001101008	AY912402

Table 2 continued

Taxon and localities	Haplotype	Size	Species-voucher[a]	AC No.
Jian'ou, Fujian	U2	1	IHB 001101006	AY912393
		1		AF499597*
	U3	1	IHB 001108001	AY912394
	U6	1	IHB 0404185	AY912398
	U8	5	IHB 001108007, 0380502, 001108005 IHB 001108010, 0404174	AY912400
	U9	1	IHB 0380503	AY912401
Shanghang, Fujian	U5	1	IHB 0404184	AY912396
	U6	1	IHB 0404172	AY912397
Zhangping, Fujian	U11	3	IHB 0404186–188	AY912403
Hejiang, Sichuan	U4	1	IHB 0404303	AY912395
Tengxian, Guangxi	U7	1	IHB 0404295	AY912399
Pseudobagrus pratti				
Xiuren, Guangxi	P1	1	IHB 0404315	AY912404
	P3	4	IHB 0404311–0404313, 0404316	AY912406
	P4	1	IHB 0404314	AY912407
Lipu, Guangxi	P2	1	IHB 0404333	AY912405
	P9	3	IHB 0404334–0404336	AY912412
Yangshuo, Guangxi	P7	1	IHB 0404286	AY912410
	P9	4	IHB 0404284-85, 0404287, 0404289	AY912413
Yongfu, Guangxi	P5	5	IHB 0404323–25, 0404327–0404328	AY912408
	P6	1	IHB 0404326	AY912409
Taoyuan, Hunan	P8	1	IHB 2003134	AY912411
Pseudobagrus truncatus				
Hechuan, Chongqing	TR1	1	IHB 0405211	AY912414
	TR2	1	IHB 0405209	AY912415
	TR3	1	IHB 0405210	AY912416
	TR4	1		AF416880*
	TR5	1	IHB 0405207	AY912417
	TR6	1	IHB 0405208	AY912418
Leiocassis crassilabris				
Mudong, Chongqing	C1	1		AF416882*
	C3	2	IHB 0405220–0405221	AY912427
Hechuan, Chongqing	C3	1	IHB 0405219	AY912426
Hejiang, Sichuan	C4	1	IHB 0404222	AY912429
Jianyang, Fujiang	C6	2	IHB 0380615, 0305221	AY912431
Jian'ou, Fujian	C7	1	IHB 0404201	AY912432
Chongzuo, Guangxi	C2	8	IHB 0380504–0380506, 0303111 IHB 0380508–09, 0303113, 0301129	AY912419
Rong'an, Guangxi	C2	1	IHB 0380614	AY912420
Liuzhou, Guangxi	C2	2	IHB 0307254–0307255	AY912421
Yongfu, Guangxi	C8	2	IHB 0404329–0404330	AY912384
	C2	5	IHB 0404337–0404341	AY912422
Yangshuo, Guangxi	C2	3	IHB 0404320–0404322	AY912423
Lipu, Guangxi	C2	1	IHB 0404318	AY912424
	C5	2	IHB 0404317, 0404319	AY912430
Zhaoping, Guangxi	C3	2	IHB 0301127–0301128	AY912425
Chenxi, Hunan	C3	1	IHB 0305243	AY912428
Leiocassis longirostris				
Mudong, Chongqing	L1	2	T020102MD	AY912440
		1		AF416889*
Hejiang, Sichuan	L2	1	IHB 0305228	AY912441
	L3	1	IHB 0305229	AY912442
Leiocassis argentivittatus				
Jinkou, Wuhan	A1	1	IHB 0305242	AY912443
	A2	1	IHB 0305240	AY912444
	A3	1	IHB 0305241	AY912445

Table 2 continued

Taxon and localities	Haplotype	Size	Species-voucher[a]	AC No.
Hemibagrus macropterus				
Mudong, Chongqing	H1	1		AF416890*
Yangshuo, Guangxi	H2	1		AF430373*
Wuyuan, Jiangxi	H3	1		AF430374*
Hemibagrus guttatus	H5	1		AF416886*
Jinxiu, Guangxi	H4	2	IHB 0305247–0305248	AY912446
Hemibagrus nemurus	H6	1		AF416882*
Pseudobagrus kyphus		1		AB085662*
Pseudobagrus aurantiacus		1		AB015989*
Pseudobagrus tokiensis		1		AB015986*
Pseudobagrus tokiensis		1		NC_004697*
Pseudobagrus nudiceps		1		AB015988*
Pseudobagrus koreanus		1		AB015991*
Pseudobagrus brevicorpus		1		AB015990*
Pseudobagrus ichikawai		1		AB015987*
Mystus sp.		1		AY458893*
Clarotes laticeps		1		AF126821*
Liobagrus anguillicauda		1		AF416888*
Silurus meridionalis		1		AF416892*

[a] IHB is the Institute of Hydrobiology, Chinese Academy of Sciences

* Asterisk (*) indicates the sequences retrieved from GenBank

three different families were used as outgroups following the procedure of Peng et al. (2005). These are: *Clarotes laticeps* (Clarotidae, accession number AF126821), *Liobagrus anguillicauda* (Amblycipitidae, AF416888), and *Silurus meridionalis* (Siluridae, AF416892).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from muscles following a standard proteinase K, phenol: chloroform extraction procedure and precipitation in isopropyl alcohol. The cyt *b* fragments was amplified and sequenced with the universal primers L14724 and H15915 (Xiao et al., 2001). The fragments were compared with the previously published complete 1138 bp cyt *b* gene of bagrids (Peng et al., 2002) to confirm that the target region was amplified by polymerase chain reaction (PCR). The reaction mixture contained 50–100 ng of template DNA, 2–3 μl of each primer, 6 μl of 10 × reaction buffer, 0.75 μl of dNTPs (10 mM), and 2.0 units of Taq polymerase in a total volume of 60 μl. The PCR profile consisted of an initial denaturation at 95°C for 3 min, and then 35 cycles of 30 s at 94°C (denaturation), 30 s at 56–59°C (annealing), 70 s at 72°C (extension), and a final extension at 72°C for 10 min.

PCR products were visualized and cut from a 0.8% low melting point agarose gel stained with ethidium bromide, then purified using the BioStar glassmilk DNA purification kit following the manufacturer's protocol. All sequences have been deposited in GenBank (accession numbers in Table 2).

Sequences analysis

Preliminary mutiple-sequence alignment was performed using Clustal X (Thompson et al., 1997) with default settings. The computer-generated alignment was then verified by eye and adjusted in SEAVIEW alignment editor (Galtier et al., 1996).

Prominent bias in base composition has been viewed as a typical feature of cyt *b* gene, accordingly base compositional biases were calculated. Nucleotide substitution patterns were analyzed using PAUP* version 4.0b10 (Swofford, 2002). Empirically, we plotted the uncorrected pairwise distance (*p*-dist) against Tamura-Nei distance (*TrN*-dist) to investigate putative substitution saturation. Comparisons among different

species were performed in MEGA v.2 (Kumar et al., 2001) using the uncorrected *p*-distance. Populations of distinct species were assigned as independent groups (results are not shown).

Phylogenetic analysis

The aligned sequences included 218 newly obtained ones and 27 retrieved sequences. Identical sequences were identified and represented in the analyses by one sequence only. A hierarchical series of likelihood ratio tests (LRTs) was implemented in the Modeltest 3.6 (Posada & Crandall, 1998) to determine the best model of evolution for neighbor-joining (NJ), maximum likelihood (ML) and Bayesian likelihood (BL) analyses. The best-fit model selected was general time-reversible (GTR, Yang, 1994), with some sites were assumed to be invariable (I) and variant sites were assumed to follow a discrete gamma (G) distribution. NJ analysis were implemented in PAUP* starting from NJ tree searches. Tree topologies were postulated using the GTR + I + G substitution model with parameters G and I estimated by Modeltest. With respect to the parsimony criterion, the most-parsimonious (MP) tree or trees were sought by using heuristic searches with starting trees obtained via the tree-bisection-reconnection (TBR) search algorithm in which taxa were added randomly with 100 iterations. To correct the possible effects of saturation, the first and second positions were weighted 3 and 5 times of the third position; meanwhile nucleotide substitution patterns were calculated in PAUP, correspondingly transitions and transversions were unequal weighted (Ti/Tv = 3/1). Degree of confidence assigned to internodes was assessed using nonparametric bootstrapping with 1,000 and 500 pseudo-replicates for NJ and MP analysis, respectively.

Using the model selected, ML trees were constructed in the software PHYML v.2.4.4 (Guindon & Gascuel, 2003) with 100 pseudo-replicates. We conducted Bayesian likelihood analysis (BL) in MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). The BL inference was conducted using the GTR + I + G model, with data matrix partitioned by codon. Starting trees were random, and phylogenetic constraints were not used. The Bayesian inference was applied to four Markov chains simultaneously each for 1.0×10^6 generations and sampled every 100th topologies. We discarded the first 1000 trees obtained before the Markov chain reached convergence. The posterior probability of the phylogeny and tree topologies were then determined from the preserved trees. A majority consensus tree was constructed from the remaining trees. This procedure was repeated two times to assure the reliability.

Tests of alternative topologies

The phylogenetic trees based on NJ, MP and BL inferences strongly support polyphyleticisms of genera *Pseudobagrus*, *Pelteobagrus* and *Leiocassis*. To further test the monophyly of these three genera, constrained trees were constructed using maximum parsimony method and implemented in PAUP*. Comparison of competing topologies, namely NJ, MP, BL and constraint trees (the ML analysis in PHYML produced a tree that was identical to the Bayesian tree, thus not included in the comparison) was carried out using the likelihood-based Kishino-Hasegawa (KH) test (Kishino & Hasegawa, 1989) and parsimony-based two-tailed Wilcoxon signed-ranks tests (Templeton, 1983), making use of 1000 bootstrap replicates with RELL (resampling estimated log-likelihood) optimization implemented in PAUP* ($P > 0.05$ in each case). Results are shown in Table 3.

Results

Cytochrome b gene nucleotide composition and substitution saturation

For the 245 sequences (outgroups included), 133 haplotypes were identified from 1092 bp fragments. No insertions/deletions were observed. Mean base composition was similar to that previously reported for bagrids (Peng et al., 2002) with an expected strong antiguanine bias (G: 7.96%, A: 32.81%, C: 34.59%, and T: 24.64%). The lower value was largely due to the instability of G content at the three codon positions: 25.82% at the first, 12.90% at the second and 2.58% at the third. Mean standard

Table 3 Two-tailed Wilcoxon signed-ranks tests and Kishino–Hasegawa (KH) test for comparison of alternative topologies

Topology	Wilcoxon signed-ranks test				Kishino–Hasegawa test		
	Maximum parsimony				Maximum likelihood		
	Tree length	*N*	*z*	*P*	– ln*L*	Diff ln *L*	*P*
MP	1903	139	– 2.8580	0.0043*	9404.0554	123.2987	0.000*
NJ	2000	130	– 8.7638	< 0.0001*	9454.0750	173.3183	0.000*
Bayesian likelihood	1857	Best			9280.7567	Best	
Pseudobagrus[a]	10230	383	– 16.9615	< 0.0001*	25982.2772	16701.5205	0.000*
Pelteobagrus[a]	9938	383	– 16.9616	< 0.0001*	24843.5213	15562.7646	0.000*
Leiocassis[a]	10082	383	– 16.9616	< 0.0001*	26341.1314	17060.3747	0.000*

* A significant difference between topologies is indicated with an asterisk

[a] Maximum parsimony trees recovered from constraint search

compositional deviations for all codon positions was 0.15. We observed the highest bias at the third codon position (0.386), intergrade at the second (0.227) and the smallest at the first position (0.02).

Among the entire 1092 bp region, 63 nucleotide positions were variable and 401 sites were parsimony informative polymorphic for MP analysis. All plotting between the uncorrected *p*-dist and the *TrN*-dist implied the substitutions were saturated in third codon positions but not in the first and second codon positions (results not shown).

Haplotypes shared by different species and interspecific mtDNA diversity

Four haplotypes (U3, U6, U8 and U10) were found to be shared by different generic species. One *Pseudobagrus ussuriensis* individual shared haplotype U10 with one *Pelteobagrus vachelli* individual. Two *Pseudobagrus ussuriensis* samples shared U6 with two *Pelteobagrus nitidus* samples. One *Pelteobagrus vachelli* sample and one *Pelteobagrus nitidus* sample shared haplotype U3 with one *Pseudobagrus ussuriensis* sample, while one *Pelteobagrus nitidus* sample and two *Pelteobagrus vachelli* samples shared U8 with five *Pseudobagrus ussuriensis* samples (see Table 2 for accession numbers).

The estimates of interspecific genetic distance showed relatively low level of genetic diversity among *Pseudobagrus*, *Pelteobagrus* and *Leiocassis* species, even among geographically distant populations (not shown). The maximum pairwise divergence value observed was 0.144 (uncorrected *p*-dist), between *Pseudobagrus pratti* and *Leiocassis argentivittatus*. The minimum pairwise divergence value was 0.017, between Chinese populations of *Pseudobagrus ondon* and Japanese sample of *Pseudobagrus aurantiacus*. Genetic divergence among *Pseudobagrus tenuis*, *Pseudobagrus ussuriensis*, *Leiocassis longirostris*, and *Leiocassis crassilabris* was 0.018–0.019. In contrast, genus *Hemibagrus* showed striking divergence with the above three genera, for the maximum divergence was 0.187, while the minimum divergence was also beyond 0.155.

Phylogenetic relationships among bagrids

Both the Two-tailed Wilcoxon signed-ranks tests and Kishino–Hasegawa (KH) test rejected NJ, MP and the constraint trees as they were significantly less consistent than the Bayesian tree (Table 3). However, most of the clades are commonly found in different tree topologies. According to the Bayesian tree, *Pseudobagrus*, *Pelteobagrus* and *Leiocassis* bagrids were mainly divided into two lineages (referred to as lineage I and lineage II in Fig. 1) except for *Leiocassis argentivittatus*, *Pseudobagrus ichikawai* and *Pseudobagrus kyphus*.

For lineage I, clade A was a robustly supported group (100% posterior probabilities) encompassing *Leiocassis crassilabris* (*C1–C8*), *Pseudobagrus ussuriensis* (*U1-U11*), *Pseudobagrus tenuis* (*TE1-TE3*) and *Leiocassis longirostris* (*L1–L3*). However, the kin-framework among these four species was weakly supported (62% and 49% Bayesian confidences) or collapsed terminal

nodes. *Pelteobagrus vachelli* (V1–V11) was closely related to clade A with high confidence (97% in Bayesian inference). *Leiocassis nitidus* (N2, collected from Russia) and *Leiocassis ussuriensis* (N5, collected from Korea) appeared in a clade which including the Chinese populations of *Pelteobagrus nitidus* with 100% posterior probabilities. This clade appeared as the sister-group of clade A plus *Pelteobagrus vachelli*, but with only 50% confidences. *Pseudobagrus pratti* (*P1–P9*) and *Pseudobagrus truncatus* (TR1-TR6) occupied the most basal position in lineage I.

Lineage II consisted of two groups. In the first one, the populations of *Pelteobagrus fulvidraco* (F1–F23) were closely affiliated to *Pelteobagrus eupogon* (E1–E10) with 100% posterior probabilities (clade B). The second group (clade C) clustered Japanese and Korean samples of *Pseudobagrus* species (*P. aurantiacus*, *P. tokiensis*, *P. nudiceps, P. koreanus*, and *P. brevicorpus*) and Chinese samples of *Pseudobagrus ondon (O1–O6)*. Noteworthily, only the sister-group relationship between *P. aurantiacus* and *P. ondon* was strongly supported (100% Bayesian confidence), and the relationships among other *Pseudobagrus* fishes in clade C did not appear so supported, since the branches uniting these bagrids were either poorly supported or essentially collapsed into polytomies (Fig. 1).

Species *Leiocassis argentivittatus* plus *Pseudobagrus ichikawai* appeared in a clade clustering with the group including lineages I and II; *Pseudobagrus kyphus* was robustly supported to be sister-group of the other bagrids except for *Hemibagrus* species (Fig. 1).

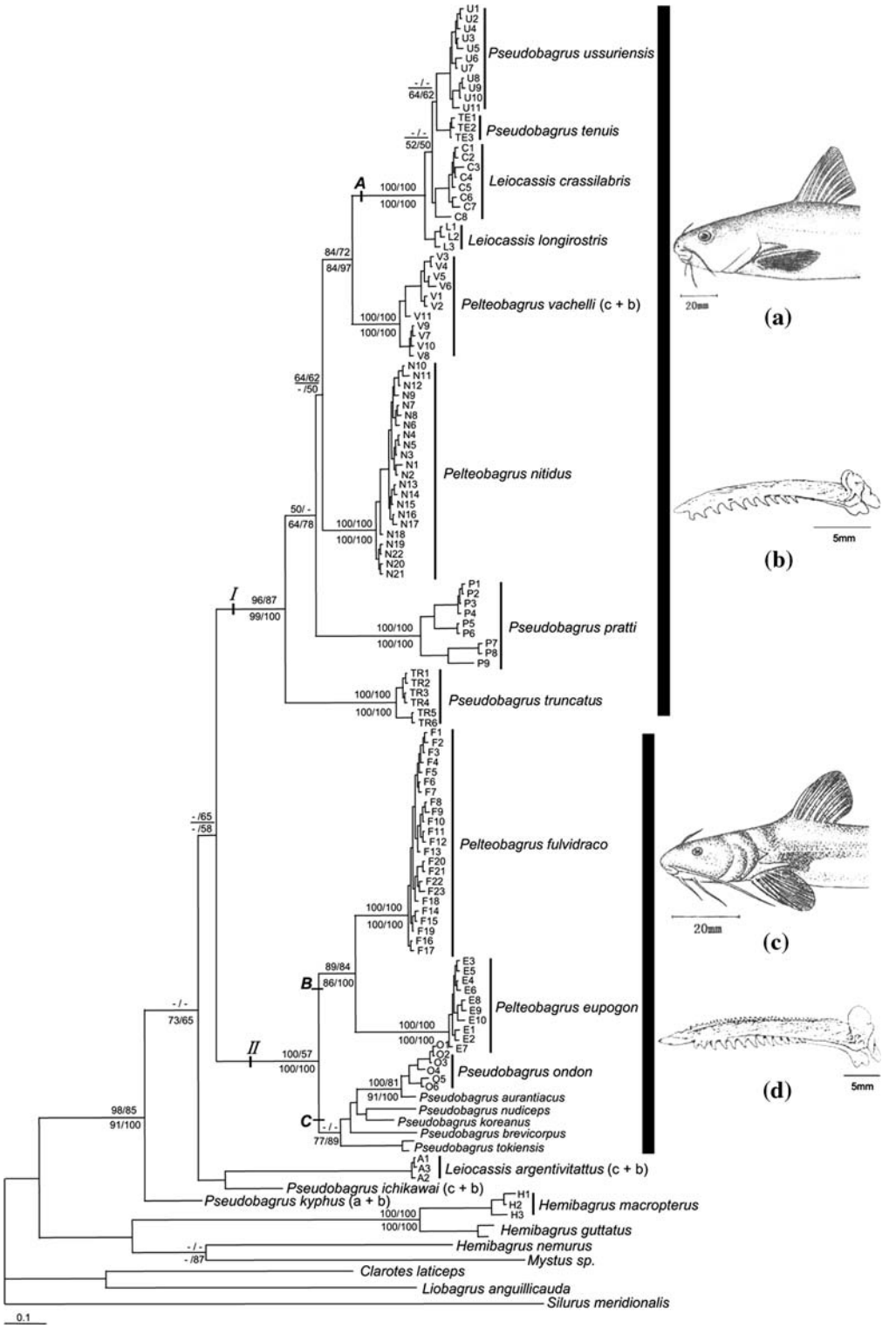
As also seen in Fig. 1, *Hemibagrus nemurus* and *Mystus* sp. were grouped, with 87% posterior probability, in a clade that appeared as the sister-group of *Hemibagrus guttatus* plus *Hemibagrus micropterus*. Thus, the phylogenetic tree obtained *Hemibagrus* appears as a non-monophyletic group.

Discussion

Polyphyly of genera *Pseudobagrus, Pelteobagrus* and *Leiocassis*

Ng (2003) comprehensively reviewed the current status of systematics of bagrid catfish. He concluded that bagrids appear to have relatively few synapomorphies that characterize the subgroups within the family which also complicated the study of their intrafamilial relationships. Our study could serve as another piece of evidence for this overview, since the phylogenetic trees do not support the monophyly of *Pseudobagrus*, *Pelteobagrus* or *Leiocassis* (Fig. 1). For genus *Pseudobagrus*, *P. ussuriensis*, *P. tenuis*, *P. pratti* and *P. truncatus* clustered with *Pelteobagrus* and *Leiocassis* fishs in lineage I while *P. ondon*, *P. aurantiacus*, *P. tokiensis*, *P. nudiceps*, *P. koreanus* and *P. brevicorpus* grouped in lineage II. For genus *Pelteobagrus*, only the sister-group relationship between *P. fulvidraco* and *P. eupogon* was strongly supported while for the *Leiocassis*, the *L. crassilabris* and *L. longirostris* samples were rather closely affiliated to *Pseudobagrus* samples than to the congeneric *L. argentivittatus*. It should be noted that Mo (1991) also pointed out that the *Leiocassis* was non-monophyletic.

The appearance of polyphyleticism can often be elucidated by the interspecific hybridization and subsequent recombination of different mtDNA lineages (e.g., Avise, 1994). Interspecific hybridization and admixture may also occur in mitochondrial genome (Goropashnaya et al., 2004a, b), which has previously been considered to be strictly of maternal inheritance without recombination and, it is not uncommon to find mitochondrial introgression. Alternatively, with respect to generic traits polyphyleticism in molecular trees was highlighted as an anticipate stage temporally intermediate to polyphyly and reciprocal monophyly, due to incomplete lineage sorting in the gene analyzed. However, it seems hard to determine which of the two hypotheses is applicable for the situation found in the East Asian bagrids. In fact, the low level of interindividual polymorphism in cyt *b* gene investigations of bagrid populations could appear, at first sight, to favor the hybridization hypothesis, since the polymorphic allele in question would be more randomly distributed among individuals under a scenario of incomplete sorting (Razafimandimbison et al., 2005). However, hybridization was mainly suggested to be responsible for nonmonophyly of sympatric species that spawn synchronously (van Oppen et al., 2001), and whether this


Pseudobagrus ussuriensis
Pseudobagrus tenuis
Leiocassis crassilabris
Leiocassis longirostris
Pelteobagrus vachelli (c + b)
Pelteobagrus nitidus
Pseudobagrus pratti
Pseudobagrus truncatus
Pelteobagrus fulvidraco
Pelteobagrus eupogon
Pseudobagrus ondon
Pseudobagrus aurantiacus
Pseudobagrus nudiceps
Pseudobagrus koreanus
Pseudobagrus brevicorpus
Pseudobagrus tokiensis
Leiocassis argentivitattus (c + b)
Pseudobagrus ichikawai (c + b)
Pseudobagrus kyphus (a + b)
Hemibagrus macropterus
Hemibagrus guttatus
Hemibagrus nemurus
Mystus sp.
Clarotes laticeps
Liobagrus anguillicauda
Silurus meridionalis
0.1
20mm
(a)
5mm
(b)
20mm
(c)
5mm
(d)

◄ **Fig. 1** Bayesian 50% majority consensus tree (– ln L = 9280.8). Numbers above nodes represent bootstrap values from NJ/MP analysis, and numbers below nodes refer to ML bootstrap proportions/BL posterior probabilities. Dashes represent nodes with bootstrap (or posterior probability) support lower than 50% or represent nodes not existed. Some populations of the same species massed on different clades with high confidences, thus values are not shown due to space restriction. *Clarotes laticeps*, *Liobagrus anguillicauda* and *Silurus meridionalis* were used as outgroups, and the tree was rooted with *Silurus meridionalis*. Figures on the right are shown to illustrate, respectively: (**a**) thin and delicate maxillary barbels that do not extend beyond proximal origin of the pectoral fins; (**b**) pectoral spines that are smooth at their anterior edges; (**c**) stout elongate maxillary barbels that extend beyond the proximal origin of the pectoral fins; (**d**) the presence of serrations on the anterior edge of the pectoral spines [figures (**b**) and (**d**) are modified from Watanabe & Uyeno, 1999]

occurs among bagrids still needs further confirmation. It should also be taken with caution that we examine only one mitochondrial marker to explore the phylogeny, for a better resolution, it will be most meaningful to add more markers, especially the nuclear genes.

The bagrid catfishes are among those siluriform groups of an older fossil record, with unambiguous fossil reports from the Eocene (Ng, 2003). Therefore, bagrids had most of the Cenozoic era to diverge (Ng, 2003). However, for East Asian group, a molecular clock calibration (Ku et al., in preparation) suggested that, most of the extant species (all the species in lineage I and lineage II) were resulted from rapid speciation within the past 10 MY. It is thus possible that the *Pseudobagrus ussuriensis*, *Pelteobagrus vachelli* and *Pelteobagrus nitidus* samples that sharing the same haplotypes did not accumulate enough base substitutions in such a relatively short time in order to be differentiated. Concerning the unresolved polytomies in the derived clades of the tree in Fig. 1, these may represent cases of explosive speciation (e.g., van Oppen et al., 2001). Thus, the generic polyphyly could probably be explained by the explosive speciation and coalescent hypotheses.

Diagnostic value of morphological traits

As referred above and summarized in Table 1, previous studies on the bagrid intrarelationships have given different results, which might reflect the use of inappropriate sampling of characters and/or taxa. In China, the original (non-cladistic) diagnoses of bagrid genera were based on length of the adipose fin, general shape of the head and the caudal fin (Chu et al., 1999). However, parts of these characters seem to be rather plesiomorphic according to their mapping in the phylogenetic tree (Fig. 1). For example, a deeply forked caudal fin is not a common character in all *Pelteobagrus* species analyzed. *Pelteobagrus eupogon* presents a moderate forked caudal fin with rounded lobes. Such morphological traits seem effectively to constitute poor phylogenetic indicators, since, as explained above, the genera *Pseudobagrus*, *Pelteobagrus* and *Leiocassis* diagnosed by them appear as non-monophyletic (Fig. 1). Thus, the taxonomy of Chinese bagrids should eagerly be the subject of detailed revision.

By contrast, a preliminary analysis of some morphological traits revealed that characters such as the length of the maxillary barbels and the shape of the pectoral spines do fit with the phylogenetic results in Fig. 1. Different states of these characters can in fact be assigned with different monophyletic assemblages in this figure: most of the fishes of lineage I possess thin and delicate barbels not extending beyond the proximal origin of the pectoral fins and only display serrations on the posterior edge of the pectoral spines; fishes of lineage II display stout, elongate barbels that extend over the proximal origin of the pectoral fins and serrations on both the anterior and posterior edges of the pectoral spines (Fig. 1). One exception is *Pelteobagrus vachelli* of lineage I, which displays smooth anterior edges of the pectoral spines but has thin maxillary barbels extending slightly over the proximal origin of the pectoral fins. But the other fishes in Fig. 1 do follow the general rule described above. This preliminary analysis of some morphological traits thus stresses that a more detailed and extensive revision of bagrid anatomy might effectively reveal useful data to reevaluate the intrarelationships of bagrid catfishes.

Monophyly of the clade including the species previously assigned to the genera *Pseudobagrus, Pelteobagrus and Leiocassis* and taxonomic suggestions

According to Peng et al. (2002), genus *Hemibagrus* occupies a basal position within East Asian bagrid catfishes. Our results support a rather basal position of *Hemibagrus macropterus*, *Hemibagrus guttatus* and *Hemibagrus nemurus* within East Asian bagrids, but the latter species appears more closely related to *Mystus* sp. than to the other two species analyzed (Fig. 1). It will thus be interesting to examine, in future works, if some of the other 37 species currently assigned to the *Hemibagrus* also appear more closely related to *Mystus* and/or other bagrid genus than to the remaining species of this genus.

Mo (1991) stated that certain Chinese species of genus *Leiocassis* (*L. longirostris*, *L. longibarbus*, *L. crassilabris*, *L. tenuifurcatus*, *L. virgatus* and *L. argentivittatus*) should in fact be assigned to genera *Pseudobagrus* and/or *Pelteobagrus*. According to Kottelat (pers. comm), the only 'true' *Leiocassis* species are those restricted to Indonesia.

Mo (1991) supported the monophyly of a clade [(*Coreobagrus*, *Pseudobagrus*) *Pelteobagrus*]. Watatanabe & Uyeno (1999) pointed out that *Coreobagrus* is not a valid genus but rather a junior synonym of *Pseudobagrus*. As can be seen in Fig. 1, according to our phylogenetic results, the genera *Pseudobagrus* and *Pelteobagrus*, as currently defined, appear as clearly non-monophyletic. Following the tree shown in Fig. 1, we suggest the synonymization of *Pelteobagrus* Bleeker 1864 with *Pseudobagrus* Bleeker 1860 (cf. Hardman, 2005) to eliminate the generic non-monophyly. Thus, in our view, all the Chinese species currently assigned to genera *Leiocassis* and *Pelteobagrus* should in fact be considered members of the genus *Pseudobagrus*.

In conclusion, our present study has focused only on the East Asian bagrid catfishes. Based on mtDNA cytochrome *b* sequence data, the currently recognized *Pseudobagrus*, *Pelteobagrus* and *Leiocassis* are found to be polyphyly. To eliminate the generic nonmonophyly, we suggest all the Chinese species currently assigned to the genera *Pelteobagrus* and *Leiocassis* should in fact be considered members of the *Pseudobagrus*. To further test the monophyly of genus *Hemibagrus*, a number of *Hemibagrus* species in the South and Southeast Asia should be included in a more comprehensive analysis.

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References

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

Chu, X., B. Zhen & D. Dai, 1999. Fauna Sinica, Class Teleostei, Siluriformes. Science Press, Beijing (in Chinese).

Dai, F., 1998. Studies on isozymes, skeleton characters of eight bagrid catfishes and comments on phylogenetic relationship (Siluriformes: Bagridae). Acta Zootaxonomica Sinica 23: 432–439 (in Chinese).

Diogo, R., 2003. Higher-level phylogeny of Siluriformes: an overview. In Kapoor, B. G., G. Arratia, M. Chardon & R. Diogo (eds), Catfishes. Science Publishers, Enfield, 353–384.

Diogo, R., 2004. Morphological evolution, aptations, homoplasies, constraints, and evolutionary trends: catfishes as a case study on general phylogeny and macroevolution. Science Publishers, Enfield, USA.

Diogo, R., P. Vandewalle & M. Chardon, 1999. Morphological description of the cephalic region of *Bagrus docmak*, with a reflection on Bagridae (Teleostei: Siluriformes) autapomorphies. Netherlands Journal of Zoology 49: 207–232.

Galtier, N., M. Gouy & C. Gautier, 1996. SEAVIEW and PHYLOWIN: Two graphic tools for sequence alignment and molecular phylogeny. Computer Applications in Biosciences 12: 543–548.

Goropashnaya, A. V., B. Fedorov, B. Seifert & P. Pamilos, 2004a. Recent speciation in the *Formica rufa* group ants (Hymenoptera, Formicidae): inference from mitochondrial DNA phylogeny. Molecular Phylogenetics and Evolution 32: 198–206.

Goropashnaya, A. V., B. Fedorov, B. Seifert & P. Pamilos, 2004b. Limited phylogeographical structure across Eurasia in two red wood ant species *Formica pratensis* and *F. lugubris* (Hymenoptera, Formicidae). Molecular Ecology 13: 1849–1858.

Guindon, S & O. Gascuel, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.

Hardman, M., 2005. The phylogenetic relationships among non-diplomystid catfishes as inferred from mitochondrial cytochrome b sequences; the search for the ictalurid sister taxon (Otophysi: Siluriformes). Molecular Phylogenetics and Evolution 37: 700–720.

Jayaram, K. C., 1968. Contributions to the study of bagrid fishes (Siluroidae: Bagridae) III. A systematic account of the Japanese, Chinese, Malayan and Indonesian genera. Treubia 27: 287–386.

Kishino, H. & M. Hasegawa, 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29: 170–179.

Kumar, S., K. Tamura, I. B. Jakobsen & M. Nei, 2001. MEGA2: molecular evolution genetics analysis software. Bioinformatics 17: 1244–1245.

van Oppen, M. J. H., J. M. Brenda., B. L. Willis & D. J. Miller, 2001. The evolutionary history of the Corals genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? Molecular Biology and Evolution 18: 1315–1329.

Maeda, H., K. Watanabe & Y. Taki, 1994. Genetic relationships of the Japanese bagrid catfishes. Journal of the Tokyo University Fisheries 81: 111–121.

Mo, T., 1991. Anatomy, relationships and systematics of the Bagridae (Teleostei: Siluroidei), with a hypothesis of Siluroid phylogeny. Theses Zoologicae 17, Koenigstein: Koeltz scientific Books.

Ng, H. H., 2003. Phylogeny and systematics of Bagridae. In Kapoor B.G., G. Arratia, M. Chardon & R. Diogo (eds), Catfishes. Science Publishers, Enfield, 439–463.

Okazaki, T., Sang-Rin Jeon, M. Watanabe & T. Kitagawa, 1999. Genetic relationships of Japanese and Korean bagrid catfishes inferred from mitochondrial DNA analysis. Zoological Science 16: 363–373.

Peng, Z., S. He & Y. Zhang, 2002. Mitochondrial cytochrome *b* sequence variations and phylogeny of East Asian bagrid catfishes. Progress in Natural Science 12: 421–425.

Peng, Z., Y. Zhang., S. He & Y. Chen, 2005. Phylogeny of Chinese catfishes inferred from mitochondrial cytochrome *b* sequence. Acta Genetica Sinica 32: 145–154.

Posada, D. & K. A. Crandall, 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14: 817–818.

Razafimandimbison, S. G., J. Moog, H. Lantz, U. Maschwitz & B. Bremer, 2005. Re-assessment of monophyly, evolution of myrmecophytism, and rapid radiation in *Neonauclea s.s*. (Rubiaceae). Molecular Phylogenetics and Evolution 34: 334–354.

Regan, C. T., 1911. The classification of teleostean fishes of the order Ostariophysi α. Siluroidae. Annals and Magazine of Natural History, London 8: 553–577.

Ronquist, F & J. P. Huelsenbeck, 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Swofford, D. L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Version 4. Sinauer, Sunderland, Massachusetts.

Templeton, A. R., 1983. Phylogenetic inference from restriction endonucleotide cleavage site maps with particular reference to the evolution of humans and apes. Evolution 37: 221–244.

Teugels, G. G., 2003. State of the art of recent siluriform systematics. In Kapoor, B. G., G. Arratia, M. Chardon & R. Diogo (eds), Catfishes. Science Publishers, Enfield, 317–352.

Thompson, J. D., T. J. Gibson & F. Plewnink, 1997. The Clustal X windows interface: flexible strategies for multiple sequences alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

Tilak, R., 1965. The osteocranium and the Weberian apparatus of the fishes of the family Bagridae (Pisces: Siluroidei). Morphologisches Jahrbuch 107: 415–443.

Watanabe, K. & T. Uyeno, 1999. Fossil bagrid catfishes from Japan and their zoogeography, with description of a new species, *Pseudobagrus ikiensis*. Ichthyological Research 46: 397–412.

Xiao, W., Y. Zhang & H. Liu, 2001. Molecular systematics of Xenocyprinae (Teleostei: Cyprinidae): taxonomy, biogeography and coevolution of a special group restricted in East Asia. Molecular Phylogenetics and Evolution 28: 473–484.

Yang, Z., 1994. Estimating the pattern of nucleotide substitution. Journal of Molecular Evolution 39: 105–111.

Zwickl, D. J. & D. H. Hillis, 2002. Increased taxon sampling greatly reduces phylogenetic error. Systematic Biology 51: 588–598.