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ScienceDirect
Molecular Phylogenetics and Evolution 48 (2008) 1223–1231
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Short Communication

A study on phylogeny and biogeography of mahseer species (Pisces: Cyprinidae) using sequences of three mitochondrial DNA gene regions

Thuy T.T. Nguyen [a,*], Uthairat Na-Nakorn [b], Srijanya Sukmanomon [b], Chen ZiMing [c]

[a] *Network of Aquaculture Centres in Asia-Pacific, Genetics and Biodiversity, PO Box 1040, Kasetsart Post Office, Bangkok 10903, Thailand*
[b] *Fish Genetics Laboratory, Department of Aquaculture, Kasetsart University, Bangkok 10900, Thailand*
[c] *School of Life Science, Yunnan University, Kunming, Yunnan, 650091, PR China*

Received 4 January 2008; accepted 8 January 2008
Available online 17 January 2008

1. Introduction

The cyprinid fishes of the three genera *Tor*, *Neolissochilus* and *Naziritor*, often referred to as mahseer, are an important group of fish. Mahseer are endemic to Asia with natural distribution encompassing the trans-Himalayan region in the northwest to Sumatra and Borneo islands in the southeast, across a number of countries such as Nepal, Pakistan, India, Sri Lanka, Myanmar, China, Thailand, Laos, Cambodia, Vietnam, Indonesia and Malaysia. These species are large-scaled barbels that live in upstream, clear, running waters (Shrestha, 1990; Ng, 2004). They are attractive as sport fish (Ng, 2004), and some species are of great economical value and of conservation concern (Nguyen et al., 2006), as well as of aquaculture potential (Haque et al., 1995; Ingram et al., 2005).

Currently 46 mahseer species are recognized, of which 23 species are of genus *Tor* Gray, 22 species belong to genus *Neolissochilus* Rainboth and one species of genus *Naziritor* Mirza (Eschmeyer et al., 2004). However, there is still much confusion with regard to taxonomy and systematics, and uniformity in diagnosis of this group. Fishes of the genus *Tor* are considered as "true mahseer" with the presence of the median lobe, as opposed to *Neolissochilus* and *Naziritor*, where the median lobe is not present. The shape, size and length of the median lobe, the features that have often been used to distinguish species of *Tor* (Zhou and Cui, 1996), are highly variable (Roberts, 1999) and are also being influenced by environmental factors, leading to confusion and as such its reliability as an indicator of species is questionable (Ng, 2004).

Molecular data have proven useful for clarifying the taxonomic relationships and defining species boundaries in morphologically conservative or highly variable groups of freshwater fish. Mitochondrial DNA (mtDNA) sequences have proven effective for elucidating phylogenetic and taxonomic relationships in many freshwater fish groups (Briolay et al., 1998; Na-Nakorn et al., 2006). However, to date there has been only one molecular genetic study which addresses question relating to genetic relationship among two Malaysian mahseer species, *T. tambroides* and *T. douronensis* (Nguyen et al., 2006) suggesting further investigation on the phylogenetic relationships within this group. In the present study, we used nucleotide sequences of three mitochondrial gene regions, large subunit ribosomal RNA (16 S rRNA), cytochrome *b* (cyt *b*) and adenosine triphosphase subunits six and eight (ATPase), from six species of *Tor* and five species of *Neolissochilus* to explore their phylogeny, evolution, and biogeography.

2. Materials and methods

2.1. Samples, DNA extraction, DNA sequencing and sequence alignment

A total of 85 individuals of 11 species belonging to two genera *Tor* and *Neolissochilus* were sampled from 2004 to 2007. Two specimens at each locality were collected and sequenced. Two individuals of *Schizothorax zarudnyi* from Sistan, Iran, were sequenced for comparative purposes. Finclips were obtained in the field and preserved in 90%

* Corresponding author. Fax: +66 2 5611727.
E-mail address: thuy.nguyen@enaca.org (T.T.T. Nguyen).

1055-7903/$ - see front matter
doi:10.1016/j.ympev.2008.01.006

ethanol then transferred to the Fish Genetics Laboratory, Kasetsart University until required. Specimens were used in the present study. Relevant information of samples are given in Table 2.

DNA was extracted using DNeasy® Tissue Kit (QIAGen Group). Partial sequences of the 16S rRNA gene were amplified using protocol as Nguyen et al. (2006). Primers used for amplifications and sequencing for the Cyt *b* and ATPase 6 genes were: L14841 (5′-AAAAAGCTTCCATCCAAC ATCTCAGCATGATGAAA-3′) and H15149 (5′-AAAC TGCAGCCCCTCAGAATGATATTTGTCCTCA-3′) (Kocher et al., 1989); and L8331 (5′-AAAGCRTTRGCC T TTTAAGC-3′) and H9236 (5′-GTTAGTGGTCAKGGG CTTGGRTC-3′) (Lovette et al., 1998); respectively. PCR was conducted at 25 μL volume, using the same mixture as described for 16S rRNA in Nguyen et al. (2006). Thermal conditions for PCR for Cyt *b* fragment were: initial denaturing 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s; and a final extension at 72 °C for 3 min, then hold at 4 °C. Thermal conditions for PCR for ATPase6 fragment were: initial denaturing 94 °C for 3 min, followed by 30 cycles of 94 °C for 45 s, 55 °C for 0:45 s and 72 °C for 1 min 30 s; and a final extension at 72 °C for 5 min, and hold at 4 °C. PCR products were stored at 4 °C. Purification and sequencing were undertaken at MacroGen Inc. (Korea). Both light and heavy strands were sequenced for each sample.

Sequences of *Cyprinus carpio* (GenBank Accession No. AP009047) (Mabuchi et al., 2006) and *S. zarudnyi* were used as outgroup. Nucleotide sequences were viewed and edited using MEGA (Kumar et al., 2004). Each gene region was aligned using Clustal X (Thompson et al., 1997) and adjusted manually using the same software. Individual sequence was deposited in GenBank. Homogeneity between gene regions was investigated using the partition homogeneity test of Farris et al. (1995) as implemented in PAUP∗ v4.0b10 (Swofford, 2001).

2.2. Phylogenetic inference

Modeltest 3.06 PPC (Posada and Crandall, 1998) was used to identify the best model of evolution for each gene region and the combined dataset. The model with the best maximumlikelihood score using the Akaike Information Criterion (AIC) was chosen (Akaike, 1973). The model best suited for the combined dataset was used in maximum-likelihood and single model Bayesian approaches; models for individual gene region were used in the mixed model Bayesian analyses.

MrBayes v3.0b4 (Ronquist and Huelsenbeck, 2003) was used to obtain Bayesian phylogeny. Different types of Bayesian Markov Chain Monte Carlo (MCMC) analyses were run to examine the effect of model choice and starting tree on the resulting parameters and likelihood scores. For each analysis we ran four Markov chains simultaneously, starting each chain from a random tree for three million generations, sampling from the chain every 10th tree.

The first analysis was run with all four genes constrained under a single model. This analysis was performed twice and is referred to as the single model analysis. The mixed model version of MrBayes allows different likelihood model parameters to be set for each partition of the data, so we also performed a mixed model analysis (four runs each), with each run started from a random tree, and refer to this as our mixed model analysis. The latter was performed under two conditions: with all parameters linked except branch lengths (unlinked branch lengths are considered proportional), and with substitution rates, character state frequencies, gamma shape parameter, and proportion of invariable sites unlinked across the four partitioned regions. These analyses are referred to as linked and unlinked mixed model analyses, respectively. Each of these analyses started with a random tree, but we ran the latter analysis a second time using the maximum-likelihood tree as the initial tree to improve the MCMC search.

Phylogenies were also estimated using the maximum-likelihood approach as implemented in PAUP∗ v4.0b10 (Swofford, 2001). Heuristic searches were performed with 10 random sequence additions and tree bisection–reconnection (TBR) branch swapping. Nodal support was assessed using the non-parametric bootstrap procedure (Felsenstein, 1985) with 100 bootstrap replicates, TBR branch swapping, and 10 random addition replicates.

A maximum parsimony tree was estimated using heuristic searches in PAUP∗ v4.0b10 (Swofford, 2001) under the same conditions as above, with gaps considered missing data. Bootstrap values were estimated using the same method as above but with 1000 bootstrap replicates.

3. Results

3.1. The dataset and sequence characteristics

The total dataset of this study includes 85 specimens, nine and 76 of which were of *Neolissochilus* and *Tor*, respectively (Table 2). Each taxon had all three gene regions sequenced and the combined dataset contained 1610 characters. Number of variable characters was 451 (including 333 parsimony-informative characters), accounting for 28.01% of the total sequence. All new sequences were deposited in GenBank under Accession Nos. EF588044–EF588204 (Table 1). In addition, six previously published 16S rRNA sequences of *T. douronensis* and *T. tambroides* of Nguyen et al. (2006) were also included in the analyses (Table 1). The partition homogeneity test revealed no significant heterogeneity between three data partitions ($P > 0.05$), allowing combination of gene regions for phylogenetic analysis.

3.2. Models of evolution

The combined dataset resulted in the best likelihood score for the transversional model with invariable sites and rate heterogeneity (TVM+I+Γ), as subset of the General Time Reversible (GTR) model. The estimated nucleo-

Table 1
Current taxonomy, haplotype codes and corresponding number of individuals for each halotype (*N*), sampling locations, and GenBank accession numbers

Species	Haplotype code	*N*	Sampling location	GenBank Accession No.		
				16S rRNA	CytB	ATPase6-8
Neolissochilus hexagonolepis (McClelland, 1839)	NHE01	2	Trishuli R., Central Region, Nepal	EF588062	EF588174	EF588118
N. soroides (Duncker, 1904)	NSO	2	Mekong R., Chiang Mai, Thailand	EF588055	EF588165	EF588109
N. stracheyi (Day, 1871)	NST	2	Chuntaburee, Eastern Thailand	EF588054	EF588164	EF588108
N. spp1	NSP1-01	1	Tegas, Lipis R., Peninsular Malaysia	EF588052	EF588162	EF588106
			Tegas, Lipis R., Peninsular Malaysia			
N. spp2	NSP2	1	Sanjiangkou, China	EF588092	EF588204	EF588148
Tor douronensis (Valenciennes, 1842)	TDO01	2	Pedamaran R., Lake Ranau, South Sumatra	EF588044	EF588149	EF588093
	TDO02	2	Asahan R., Lake Toba, North Sumatra	EF588077	EF588189	EF588133
	TDO03	1	Kerinci, Jambi, Sumatra	EF588074	EF588186	EF588130
	TDO04	1	Kerinci, Jambi, Sumatra	EF588075	EF588187	EF588131
	TDO05	2	Limbang R., Sarawak, East Malaysia	EF588046	EF588151	EF588095
	TDO06	1	Moyog R., Penampamg, Sabah, East Malaysia	EF588045	EF588150	EF588094
	TDO07	1	Moyog R., Penampamg, Sabah, East Malaysia	EF588073	EF588185	EF588129
	TDO08	2	Wario R., Kota Belud, Sabah, East Malaysia	EF588059	EF588171	EF588115
	TDO09	2	Bunan R., Sarawak, East Malaysia	AY973167[a]	EF588168	EF588112
	TDO10	1	Layar R., Sarawak, East Malaysia	AY973167[a]	EF588152	EF588096
	TDO11	1	Layar R., Sarawak, East Malaysia	AY973167[a]	EF588153	EF588097
	TDO12	1	Rejang R., Sarawak, East Malaysia	AY973166[a]	EF588154	EF588098
	TDO13	1	Rejang R., Sarawak, East Malaysia	AY973166[a]	EF588155	EF588099
	TDO14	1	Srepok R., Daklak, Vietnam	EF588078	EF588190	EF588134
	TDO15	1	Srepok R., Daklak, Vietnam	EF588079	EF588191	EF588135
	TDO16	1	Menghan, Yunnan, China	EF588082	EF588194	EF588138
	TDO17	1	Menghan, Yunnan, China	EF588083	EF588195	EF588139
	TDO18	2	Langcangjiang R., upper Mekong, Yunnan, China	EF588049	EF588159	EF588103
	TDO19	2	Menglun, Yunnan, China	EF588084	EF588196	EF588140
	TDO20	2	Jinghong, Yunnan, China	EF588086	EF588198	EF588142
T. khudree (Sykes, 1839)	TKH01	2	Lonovaa, Maharashtra State, India	EF600900	EF588156	EF588100
	TKH02	2	Chalakudy R., Western Ghats, Kerala, India	EF588070	EF588182	EF588126
	TKH03	2	Periyar Lake, Periyar Tiger Reserve, Thekkadi, Kerala, India	EF588069	EF588181	EF588125
	TKH04	2	Akkaraseeyawatta, Kuruwita, Sri Lanka	EF588047	EF588157	EF588101
	TKH05	2	Bopathella, Ratnapura district, Sri Lanka	EF588048	EF588158	EF588102
T. macrolepis (Heckel, 1838)	TMA01	1	Indus R., Pakistan	EF588091	EF588203	EF588147
T. putitora (Hamilton, 1822)	TPU01	1	Lake of Pokhara, Western Region, Nepal	EF588088	EF588200	EF588144
	TPU02	1	Lake of Pokhara, Western Region, Nepal	EF588089	EF588201	EF588145
	TPU03	2	Koshi R., Western Region, Nepal	EF588090	EF588202	EF588146
	TPU04	2	Kali Gandaki R.,Western Region, Nepal	EF588061	EF588173	EF588117
	TPU05	2	Trishuli R. Central Region, Nepal	EF588060	EF588172	EF588116
	TPU06	2	Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh, Bangladesh	EF588071	EF588183	EF588127
T. tambroides (Bleeker, 1854)	TTA01	2	Phetchaburi Province, Thailand	EF588057	EF588167	EF588111
	TTA02	2	Kachanaburi, Thailand	EF588087	EF588199	EF588143
	TTA03	1	Nenggiri R., Kelantan, Peninsular Malaysia	EF588051	EF588161	EF588105
	TTA04	1	Nenggiri R., Kelatan, Peninsular Malaysia	EF588050	EF588160	EF588104
	TTA05	2	Narathivat Province, Thailand	EF588056	EF588166	EF588110
	TTA06	2	Lipis R., Pahang, Peninsular Malaysia	EF588058	EF588170	EF588114
	TTA07	2	Kelantan R., Kelantan, Peninsular Malaysia	EF588053	EF588163	EF588107

(*continued on next page*)

Table 1 (*continued*)

Species	Haplotype code	*N*	Sampling location	GenBank Accession No.		
				16S rRNA	CytB	ATPase6-8
	TTA08	2	Limbang R., East Malaysia	AY973157[a]	EF588169	EF588113
	TTA09	1	Lipis R., Raub, Pahang, Peninsular Malaysia	EF588063	EF588175	EF588119
	TTA10	2	Menglun, Yunnan, China	EF588085	EF588197	EF588141
	TTA11	1	Terrenganu, Peninsular Malaysia	EF588064	EF588176	EF588120
	TTA12	1	Terrenganu, Peninsular Malaysia	EF588065	EF588177	EF588121
	TTA13	2	Kerinci, Jambi, Sumatra	EF588076	EF588188	EF588132
	TTA14	1	Salween R., Mae Hong Son, Thailand	EF588066	EF588178	EF588122
	TTA15	1	Salween R., Mae Hong Son, Thailand	EF588067	EF588179	EF588123
T. tor (Hamilton, 1822)	TTO01	2	Kali Gandaki R., Nepal	EF588068	EF588180	EF588124
	TTO02	2	Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh, Bangladesh	EF588072	EF588184	EF588128
Schizothorax zarudnyi (Nikolskii, 1897)	SZA1	1	Sistan, Iran	EF588080	EF588192	EF588136
	SZA2	1	Sistan, Iran	EF588081	EF588193	EF588137

[a] Sequences obtained from Nguyen et al. (2006).

tide frequencies were: A = 0.3210, C = 0.2850, G = 0.1561 and T = 0.2379. The substitution model incorporated the following rate matrix: [A–C] = 3.3009, [A–G] = 52.6708, [A–T] = 2.1718, [C–G] = 3.2982, [C–T] = 52.6708, [G–T] = 1.0000. The shape parameter of the discrete gamma distribution was Γ = 0.6383 with the proportion of invariable sites of 0.5404. The models of evolution of the individual gene regions were: 16S rRNA–GTR+I, ATPase6–GTR+I+Γ, and Cyt *b*–K81uf+I+Γ. These models were used to determine the number of substitution types and the inclusion of gamma rate distribution and/or proportion of invariable sites in the Bayesian analyses.

3.3. Phylogenetic relationships among samples

Identical tree topologies (Fig. 1) were recovered from all methods of analysis, including single model and the unlinked mixed model Bayesian analysis, and the MP and ML analysis. The tree was bifurcated into different lineages, corresponding to *Neolissochilus* and *Tor*, with absolute support (1.00 posterior probabilities (pP), 100% bootstrap support from MP and ML). Average level of divergence between the two genera was 0.072 (Table 2) (see Fig. 2).

All samples of *Neolissochilus* were grouped together with moderate support. The remaining samples formed the second lineage, corresponding to genus *Tor* with low to moderate support (pP = 0.69, 50% and <50% bootstrap values from MP and ML analysis, respectively). Relationships among *Tor* species were not well resolved, especially with the position of *T. douronensis* in relation to other species.

Samples of *T. douronensis* formed three distinct lineages with strong support (pP = 1.00, 84–100% and 73–100% bootstrap supports resulted from MP and ML analysis, respectively). These three lineages corresponded well with three isolated geographic areas, these being the Mekong river system from China to Vietnam, Sumatra Island in Indonesia, and Sarawak and Sabah (Borneo Island) in East Malaysia, in which the Mekong and Sumatra lineages appeared to be more closely related, although with moderate support.

Three species *T. tambroides*, *T. khudree* and *T. tor* were clustered into one group with moderate support. Within *T. tambroides*, two distinct lineages were observed, one of which was restricted to the Salween River in Thailand. Average nucleotide divergence between these two lineages was 0.017. Samples of *T. khudree* from Sri Lanka were distinct from that of Indian samples, with average level of divergence of 0.046. Sample of *T. macrolepis* is clustered with the *T. putitora* group with almost absolute posterior probability (pP=0.99).

4. Discussion

4.1. Taxonomic classifications

Nearly all previous attempts to estimate relationships between species of mahseer have been based on only mor-

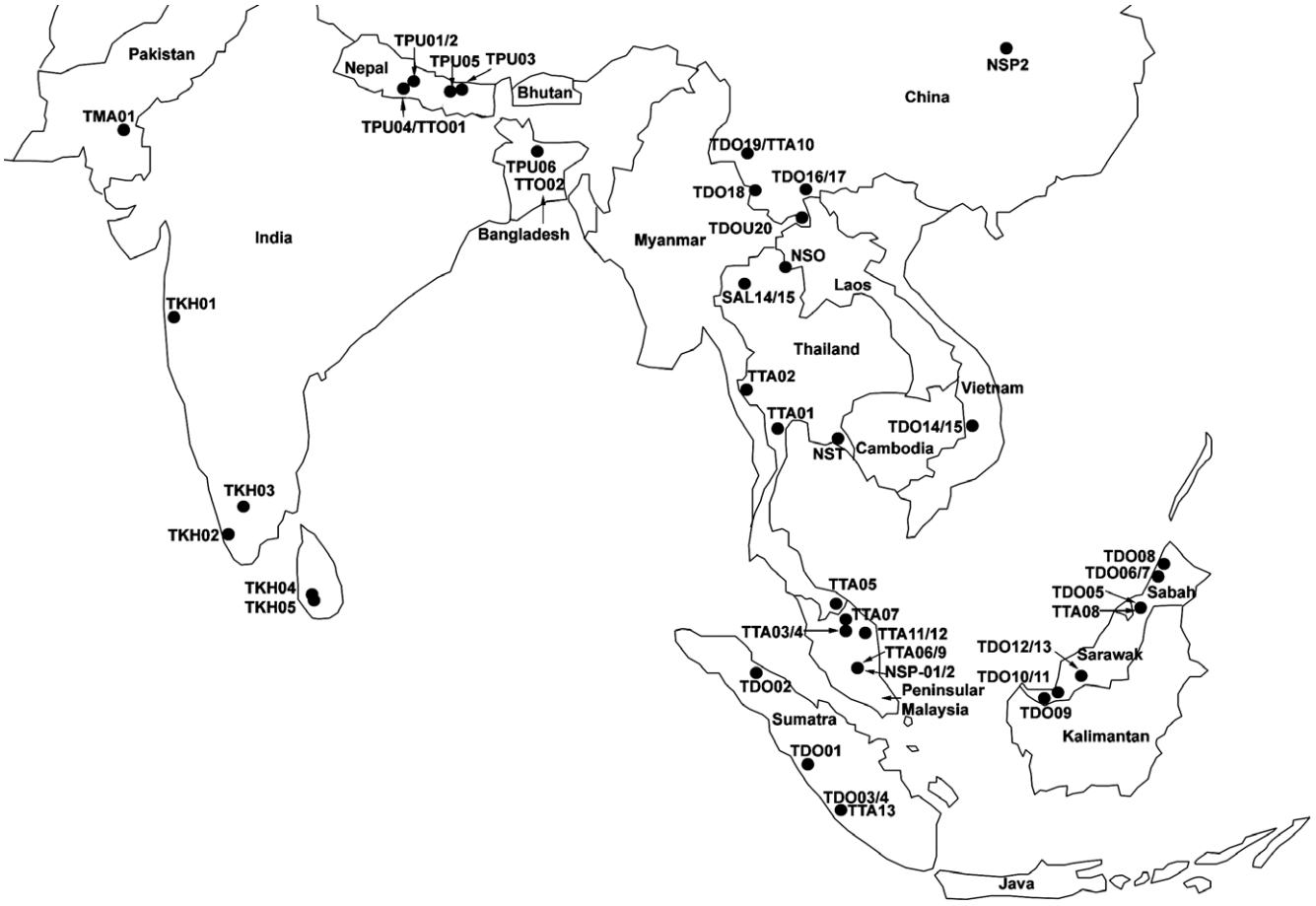

Fig. 1. Map showing sampling locations of *Tor* and *Neolissochilus* samples analysed in the present study.

Table 2
Levels of nucleotide divergence within and between 11 mahseer species, and that with the outgroup *S. zarudnyi*

Species	1	2	3	4	5	6	7	8	9	10	11	12
1. *T. tambroides*	0.010											
2. *T. khudree*	0.035	0.035										
3. *T. tor*	0.025	0.041	0.026									
4. *T. douronensis*	0.052	0.065	0.063	0.048								
5. *T. macrolepis*	0.037	0.051	0.044	0.062	—							
6. *T. putitora*	0.031	0.044	0.039	0.055	0.009	0.003						
7. *N.* spp1	0.077	0.083	0.086	0.091	0.079	0.072	0.001					
8. *T. soroides*	0.068	0.076	0.076	0.088	0.073	0.066	0.018	0.000				
9. *N. stracheyi*	0.062	0.074	0.072	0.086	0.069	0.061	0.029	0.025	0.000			
10. *N.* spp2	0.058	0.070	0.063	0.079	0.058	0.053	0.056	0.051	0.054	0.000		
11. *N. hexagonolepis*	0.058	0.074	0.066	0.085	0.075	0.067	0.082	0.071	0.060	0.065	0.000	
12. *S. zarudnyi*	0.205	0.225	0.217	0.224	0.198	0.195	0.242	0.238	0.235	0.235	0.250	0.001

The estimates were based on the best suited model determined by Modeltest.

phological characters. After a long period of time since the first description of *Tor* (Gray, 1833), Rainboth (1985) erected the new genus *Neolissochilus* based on the absence of a median lobe and other characters such as gill raker counts and pharyngeal arches. In the present study, all species within *Neolissochilus* formed a monophyletic group with a posterior probability of 0.81, and MP and ML bootstrap supports of 76% and 72%, respectively; indicating *Neolissochilus* is a sister genus of *Tor* and that they are sufficiently different to warrant generic status. However, monophyletic status of these must be cautiously interpreted because not all described species were examined in the present study.

Samples of *Neolissochilus* spp1 collected from Pahang river system in Peninsular Malaysia are often considered as *T. soro* (Valenciennes, 1842), a species that was first described from river Sading Vetang, Bantam, Java, Indonesia as *Barbus soro*, and later grouped under *Neolissochilus*.

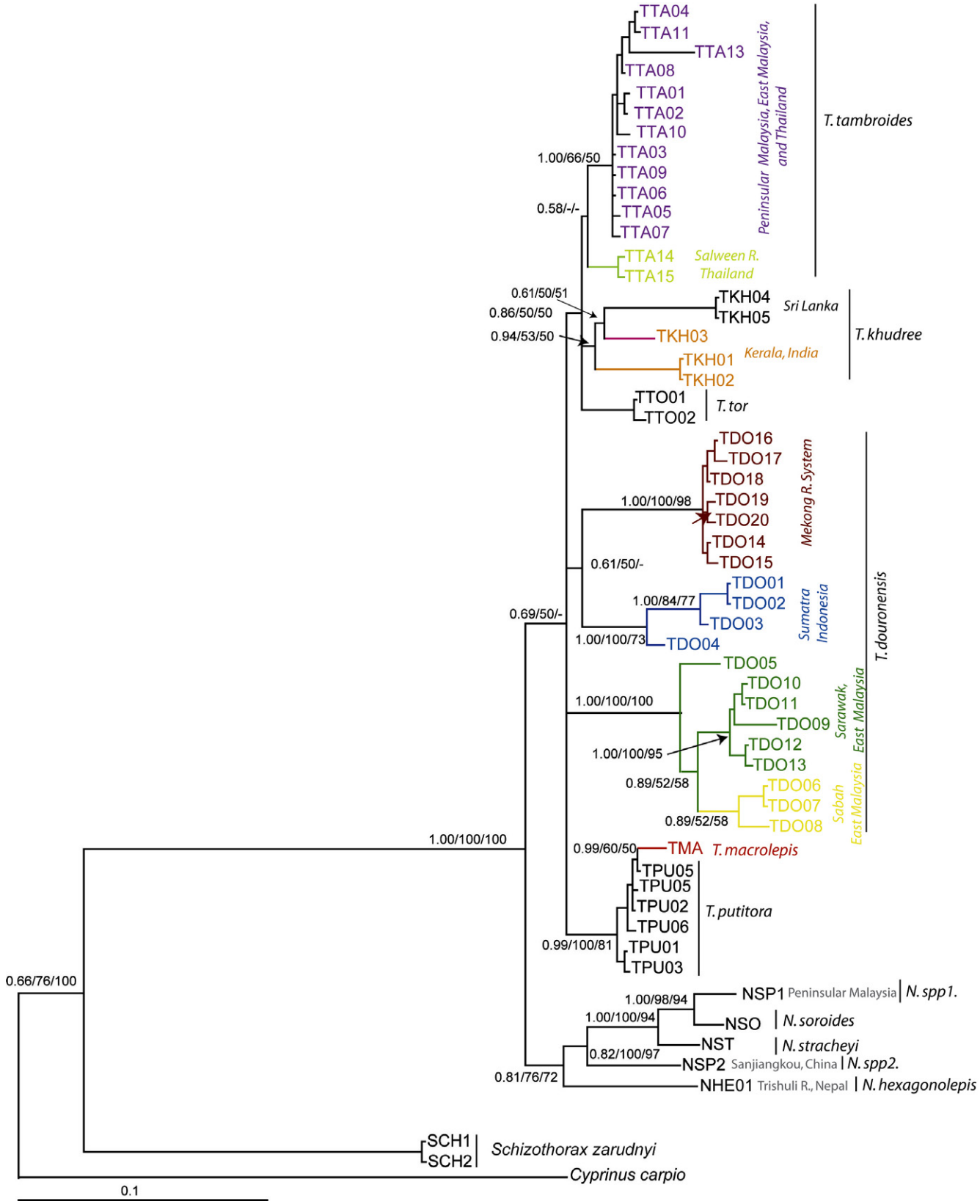

Fig. 2. Bayesian consensus tree after burnin calculated using unlinked mixed models. Clade posterior probabilities are shown at each node followed by bootstrap values from maximum parsimony and maximum-likelihood analyses from the same dataset, respectively.

Kottelat (2000) thought *T. soro* was a valid species, and some regarded it as a junior synonym of *T. tambra* (Lim et al., 1990; Roberts, 1993). Roberts (1999) suggested *T. soro* may belong to the genus *Neolissochilus* Rainboth

as it does not have a median lobe. As the holotype of *B. soro* is likely lost (Roberts, 1993) and as such there are no means for comparison of the specimens collected from the present study, the valid species name is not certain at this stage. Nevertheless, findings from the present study indicate that the characters described by Rainboth (1985), including, the presence/absence of the median lobe can be used as a character to distinguish *Neolissochilus* and *Tor*, and that the specimens of collected herein belong to the former.

Within the genus *Tor*, this discussion starts with *T. khudree* group. Samples from Sri Lanka and India were clustered together with high level of support. Though *T. khudree* samples from Sri Lanka were previously recognized as *T. longispinus*, and subsequently treated as a subspecies of *T. khudree longispinus* (Talwar and Jhingran, 1991), most authors accept that they are synonym of *T. khudree* (Pethiyagoda, 1991; Menon, 1992), and the present findings support the latter if phylogenetic species concept is applied.

Tor tambroides is distributed widely and has been reported to occur in Chao Phraya, Salween and Mekong basins, Peninsular Malaysia, Sumatra, Java, and Borneo (Eschmeyer et al., 2004). Samples of *T. tambroides* obtained from the Mekong and Salween river basins, Peninsular Malaysia, and Sarawak formed a monophyletic clade. Roberts (1999) examined previous collections of *T. tambroides* from Pahang, Johor and Terengganu in Peninsular Malaysia, Bintulu in Sarawak and indicated that fish with long median lobes could be tentatively identified as *T. tambroides*. Sequences reported herein are from specimens that have a long median lobe, and include samples from Pahang river system (TTA06 and TTA09), indicating that *T. tambroides* is a valid species although Roberts (1999) suggested it might be a junior synonym of *T. tambra* (Valenciennes, 1842).

Tor macrolepis Heckel was first described from specimens from Kashmir as *Labeobarbus macrolepis*. Later on it was accepted as *Barbus macrolepis* Gunther. However, Silas (1960) suggested that this species is a junior synonym of *T. putitora*. Subsequently, Mirza and Bhatti (1996) recognized the Indus golden mahseer as a distinct subspecies, i.e. *T. putitora macrolepis*. In a recent revision, Mirza (2004) compared morphological characters of 142 specimens collected from Pakistan and Azad Kashmir and suggested that the golden mahseer in the Indus river system in Pakistan deserved its species status and be referred to as *T. macrolepis*. Evidences from the present study support the view that if there is only one species of golden mahseer in the Indus river system then it should be *T. putitora*.

Taxonomy of *T. douronensis* appeared to be the most complicated. Two distinct lineages corresponding to two isolated geographic regions, i.e. the Borneo Island and the Mekong river system + Sumatra Island, were observed. Average nucleotide divergence of 0.068 between these lineages indicates at least two species in this group. According to previous descriptions (Roberts, 1993; Kottelat, 2001; Parenti and Lim, 2005), samples from the Mekong river system + Sumatra Island are more likely to be *T. tambra*, and those of the Borneo Island are *T. douronensis*. It is noted that type specimens of both *T. douronensis* and *T. tambra* were from Java Island of Indonesia. High level of divergence (0.053) between the two geographically isolated lineages in the Mekong and Sumatra Island might also deserve species status, or at least should be identified at the subspecies level.

4.2. Biogeographical history of mahseers

Findings from the present study suggest two possible hypotheses concerning the formation of the current species distribution of mahseers: vicariance fragmentation and range expansion. Allopatric distribution of *Neoplissochilus* and *Tor* indicates these genera have co-existed for a significant period of time. If it is assumed that mitochondrial genes in mahseers evolve at a similar rate to other freshwater fish, i.e. 1–2% per nucleotide per million years, then the division between *Neolissochilus* and *Tor* probably occurred 7.6–3.8 Mya, or in the Miocene epoch.

If the same rate of mutation is applied, most speciation events within the genus *Tor* were estimated to occur in the late Miocene or early Pleistocene (6.5–1.25 Mya). It is interesting that two contrasting phylogeographical patterns were observed in two sympatrically distributed species, *T. douronensis* and *T. tambroides*. Data from the present study and that of Nguyen et al. (2006) and Nguyen (in press) revealed high levels of genetic variation and clear patterns of population structure in the former, while little variation and no apparent structure were observed in the latter.

The exposure of the Sunda Shelf, the extended continental shelf that connects the islands of Sumatra and Borneo to the Southeast Asian mainland, in the Pleistocene epoch (1.6–0.1 Mya) (Voris, 2000), is thought to have influenced on altering the river courses in Southeast Asia, and therefore played an important role in dispersal ability and expansion range of many freshwater species (McConnell, 2004). Present data indicate that the three geographically isolated lineages of *T. douronensis* separated in late Miocene and as such the Sunda Shelf seems to have not mediated migration of these lineages between the Borneo and Sumatra islands, and the mainland Southeast Asia. The present observation on high levels of divergence of three lineages within *T. douronensis* suggests that they would have separated prior to Pleistocene rather than as a result of dispersal during recent low sea levels in the Pleistocene. This is also true in the case of intraspecific divergence, as observed in *Hemibagrus nemurus* (Dodson et al., 1995) and *Barbodes gonionotus* (McConnell, 2004). Dodson et al. (1995) and McConnell (2004) reported that little exchange between genetic groups of *these* two species has occurred between the mainland and the Sunda Islands during recent glaciations.

In contrast, low levels of variation within *T. tambroides* over its distribution range indicate that it may have

achieved its wide distribution in a recent time, probably in relation to the exposure of the Sunda Shelf. Low levels of divergence between haplotypes in different islands currently separated by sea indicate some level of migration between populations during periods of low Pleistocene sea levels, or recent dispersal of the species. During Pleistocene glacial maxima, the sea level was lower than at present and the Islands of the Sunda Shelf (Sumatra, Boneo and Java) and the Asian mainland were connected by lowlands traversed by rivers, which could play an important role for dispersal or range expansion of freshwater species.

5. Conclusions

This is the first attempt to reconstruct a robust phylogeny of the mahseer group. When comparing methods of phylogenetic reconstruction, partitioning and modeling data by individual mtDNA gene regions produced identical results to analyses with a single model applied to the entire dataset and these results were very similar to those using parsimony and maximum-likelihood. The findings from the present study provide useful insights into taxonomic status of mahseer, and set the stage for future investigations dealing with phylogeography, taxonomy, conservation, and coevolution within this interesting and important group of fish. Inclusion of additional taxa would warrant further investigations.

Acknowledgments

The authors thank Prof. Sena S. De Silva, Director General of the Network for Aquaculture Centres in Asia-Pacific, Honorary Professor, Deakin University, Australia, for initiating this work and without whose support this study would have not been completed. A number of colleagues helped in sample collection, and we thank them for their kind assistance. Financial support for the project was from a grant to Prof. Sena S. De Silva from the State Government of Sarawak, Malaysia.

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