

Short communication

Uplift of the Tibetan plateau: Evidence from divergence times of glyptosternoid catfishes

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1. Introduction

The Tibetan plateau is the largest and highest plateau on Earth, covering more than 5 million square km at an average elevation of over 5 km. Its geomorphology and climate show substantial interregional variation, leading to remarkable faunal and floral diversity and high levels of endemism. Much of the elevation of the plateau derives from the collision of India and Eurasia around 50–45 million years (Ma) ago. The Cenozoic uplift processes of the Tibetan plateau and its environmental effects are of considerable scientific interest, but the mechanism, amplitude, and timing of the uplift are still controversial (Li and Fang, 1999; Tapponnier et al., 2001). Studies of molecular phylogeny and biogeography of organisms endemic to the plateau offer an alternative approach for investigating the historical processes of the geological events and the concomitant ecological changes.

Paleobiogeographic analyses of freshwater fishes can provide a link between the geological and biotic evolution of the Tibetan plateau, because their dispersal depends on the formation of direct connections between drainages (Bermingham and Martin, 1998; Lundberg, 1993); the history of these interconnections reflects the underlying geology. Glyptosternoid catfishes present an ideal group for investigating the history of the plateau because of their limited distribution among the drainages of southern Tibet and east Himalaya, a distribution pattern that is likely to have been influenced significantly by the tectonic movements during the strong uplift of the Tibetan plateau.

We estimate the phylogeny and divergence times of extant glyptosternoid catfishes from nucleotide sequences of one nuclear and two mitochondrial genes to produce a comprehensive in situ view of glyptosternoid evolution within the Tibetan plateau. Several different relaxed molecular clock methods, including penalized likelihood and Bayesian analysis, are used to estimate divergence dates. These date estimates are then placed in the context of geological and climatological events, with a view to identifying possible correlations between biotic and abiotic phenomena. Our findings are consistent with a significant uplift of the Tibetan plateau in the Late Miocene extending to the Pliocene.

2. Materials and methods

The sisorid specimens, sampled in the southern Tibetan Plateau and east Himalaya (Table 1; Fig. 1), consisted of 25 individuals from 12 species, including 19 individuals from eight glyptosternoid species and six individuals from four non-glyptosternoid sisorid species. Due to difficulties in sampling, two genera (*Myserglanis* and *Parachiloglanis*) were not included in this study. The nonsisorid outgroups, akysids (*Akysis brachybarbatus*), amblycipitids (genera *Lio-bagrus* and *Xiurenbagrus*), and silurids (*Wallago attu*), were included to ensure proper rooting of the phylogeny.

Total DNA was extracted from muscles according to the phenol/chloroform extraction procedure (Sambrook et al., 1989). The target region (mitochondrial *cytb* and *ND4* genes, as well as nuclear *rag2* gene) was amplified from the total DNA extracts using the polymerase chain reaction (PCR) with the primers adapted from Ding et al. (2005), Hardman and Page (2003), and Xiao et al. (2001). The PCR reaction contained approximately 100 ng of template DNA,

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Table 1
GenBank accession numbers for sequences employed in this study

Taxa	Locality	Voucher No.	GenBank Accession No.		
			cyt b	ND4	RAG2
<i>Bagarius yarrelli</i>	Tengchong, Yunnan	IHB 0106288	AF416897	DQ192407	DQ192431
<i>Glyptothorax fokiensis</i>	Leshan, Sichuan	IHB 0305142	DQ192467	DQ192408	DQ192430
<i>Gagata cenia</i>	Manhaiqiao, Yunnan	IHB 0411128	DQ192468	DQ192409	DQ192432
<i>Pseudecheneis sulcatus</i>	Yangbi, Yunnan	IHB 2003230	AF499601	—	DQ192453
<i>Pseudecheneis sulcatus</i>	Beibeng, Tibet	IHB 0305111	DQ192469	DQ192412	DQ192451
<i>Pseudecheneis sulcatus</i>	Beibeng, Tibet	IHB 0305112	DQ192470	DQ192413	DQ192452
<i>Glyptosternon maculatum</i>	Lhasa, Tibet	IHB 2003115	DQ192471	DQ192400	DQ192445
<i>Glyptosternon maculatum</i>	Lhasa, Tibet	IHB 2003116	DQ192472	DQ192402	DQ192447
<i>Glyptosternon maculatum</i>	Lhasa, Tibet	IHB 2003117	DQ192473	DQ192401	DQ192446
<i>Pareuchiloglanis kamengensis</i>	Manhaiqiao, Yunnan	IHB 0411123	DQ192477	DQ192419	DQ192441
<i>Pareuchiloglanis kamengensis</i>	Manhaiqiao, Yunnan	IHB 0411125	DQ192476	DQ192418	DQ192442
<i>Pareuchiloglanis kamengensis</i>	Chayu, Tibet	IHB 0305091	DQ192474	DQ192420	DQ192439
<i>Pareuchiloglanis kamengensis</i>	Chayu, Tibet	IHB 0305094	DQ192475	DQ192421	DQ192440
<i>Glaridoglanis andersonii</i>	Chayu, Tibet	IHB 0305171	AY601769	DQ192404	DQ192449
<i>Glaridoglanis andersonii</i>	Chayu, Tibet	IHB 0380484	DQ192460	DQ192403	DQ192450
<i>Exostoma labiatum</i>	Guyong, Yunnan	IHB 2003235	AF499598	DQ192405	—
<i>Exostoma labiatum</i>	Beibeng, Tibet	IHB 0305174	DQ192461	DQ192406	DQ192448
<i>Oreoglanis macropterus</i>	Gulang, Yunnan	KIZ 200405007	DQ192479	DQ192423	DQ192444
<i>Euchiloglanis davidi</i>	Amo, Yunnan	IHB 0406888	DQ192485	DQ192429	DQ192438
<i>Pseudexostoma yunnanensis</i>	Guyong, Yunnan	IHB 2003234	DQ192478	DQ192422	DQ192443
<i>Pareuchiloglanis sinensis</i>	Mabian, Sichuan	IHB 0408003	DQ192480	DQ192428	DQ192433
<i>Pareuchiloglanis sinensis</i>	Mabian, Sichuan	IHB 0408004	DQ192483	DQ192424	DQ192435
<i>Pareuchiloglanis sinensis</i>	Mabian, Sichuan	IHB 0408005	DQ192481	DQ192426	DQ192434
<i>Pareuchiloglanis sinensis</i>	Mabian, Sichuan	IHB 0408006	DQ192484	DQ192427	DQ192436
<i>Pareuchiloglanis sinensis</i>	Mabian, Sichuan	IHB 0408008	DQ192482	DQ192425	DQ192437
<i>Liobagrus marginatus</i>	Hejiang, Sichuan	IHB 0404263	DQ192462	DQ192414	DQ192454
<i>Liobagrus anguillicauda</i>	Chongan, Fujian	IHB 0404266	DQ192463	DQ192415	DQ192455
<i>Xiurenbagrus xiurenensis</i>	Xiuren, Guangxi	IHB 0404027	DQ192464	DQ192416	DQ192456
<i>Xiurenbagrus xiurenensis</i>	Xiuren, Guangxi	IHB 0404020	DQ192465	DQ192417	DQ192457
<i>Akysis brachybarbatus</i>	Menglun, Yunnan	IHB 0405037	DQ192466	DQ192410	DQ192458
<i>Wallago attu</i>	Menglun, Yunnan	IHB SI3111	AF477828	DQ192411	DQ192459

1 μ L of each primer, 5 μ L of 10 \times reaction buffer, 2 μ L dNTPs (each 2.5 mM), and 2.0 U of *Taq* DNA polymerase in total 50 μ L volume. The reactions were cycled at the following temperatures: an initial 94 $^{\circ}$ C denaturation for 3 min, 30 cycles of 94 $^{\circ}$ C denaturation for 45 s, 50–60 $^{\circ}$ C annealing for 45 s, 72 $^{\circ}$ C extension for 1 min, and a final 72 $^{\circ}$ C extension for 5 min. Amplified DNA was fractionated by electrophoresis through 0.8% low-melting agarose gels, recovered from the gels, and purified using BioStar Glass-milk DNA purification Kit according to manufacturer's instructions. Purified DNA was sequenced with the Perkin-Elmer BigDye DNA Sequencing Kit according to the manufacturer's protocol with the primers used in PCR. The sequences have been deposited in GenBank (accession numbers listed in Table 1).

Two mitochondrial genes (*cytb* and *ND4*) were obtained, yielding alignments of 1137 and 615 bp, respectively. One nuclear gene, *rag2*, was partially sequenced (906 bp). The three genes were concatenated to form a combined data set of 2658 aligned sites.

Phylogenetic analysis was performed using maximum parsimony and maximum likelihood using PAUP* v4b10 (Swofford, 2002), and Bayesian inference using MrBayes v3.0 (Huelsenbeck and Ronquist, 2001). The general time-reversible model of nucleotide substitution was used in the

maximum likelihood and Bayesian analyses, with γ -distributed rates among sites and a proportion of invariant sites (the GTR+ Γ +I model). To assess statistical support for hypothesized clades, bootstrap analysis was performed with 1000 and 100 replicates for the maximum parsimony and maximum likelihood analyses, respectively. In the Bayesian analysis, posterior distributions were obtained by Markov chain Monte Carlo (MCMC) analysis with one cold chain and three heated chains. Samples of trees and parameters were drawn every 100 steps from a total of 1,000,000 MCMC steps.

To investigate the behaviour of rates throughout the tree, Bayesian analysis was performed using the program BEAST v1.3 (Drummond and Rambaut, 2005), under a relaxed clock model with branch-specific rates following a lognormal distribution. Posterior estimates were obtained by sampling every 100 MCMC steps from a total of 1,000,000 steps. The coefficient of variation of rates was found to be 2.0×10^{-3} (95% highest posterior density (HPD) $5.7 \times 10^{-7} - 5.8 \times 10^{-3}$), suggesting limited departure from a molecular clock (a condition in which the coefficient of variation equals zero). The rate covariance was 0.61 (95% HPD 0.57–0.64), which indicates a moderate amount of rate autocorrelation among adjacent branches in the tree. Accordingly, divergence date estimation was

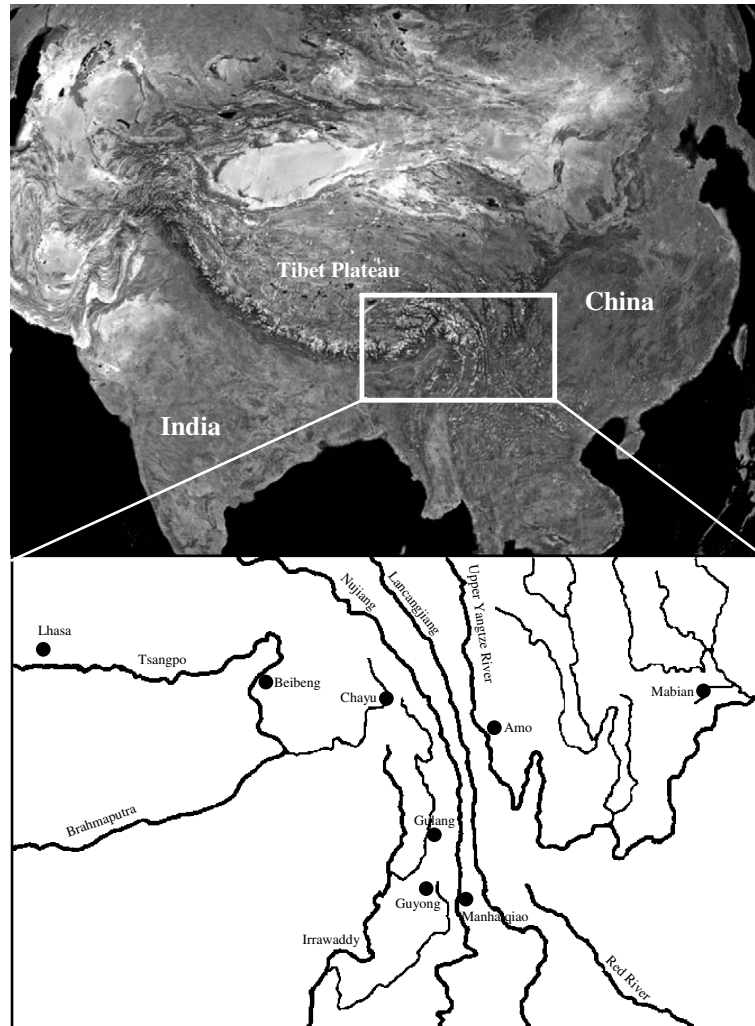


Fig. 1. Map of the Tibetan plateau showing the locations of glyptosternoid catfishes.

performed using autocorrelated relaxed clock models, implemented in the programmes *multidivtime* (Thorne et al., 1998) and *PhyBayes* (Aris-Brosou and Yang, 2002). For both of these programs, a lognormal model of rate variation was assumed. Divergence dates were also estimated using penalized likelihood, as implemented by the program *r8s* (Sanderson, 2002), with an optimized smoothing parameter of 10^{-4} as determined by a cross-validation analysis with the TN algorithm. Estimates were calibrated using three age constraints (C1, C2, and C3; Fig. 2). The C1 calibration point is based on the fossil record of *Bagarius yarrelli* from the Pliocene (5.3–1.8 Ma ago) of the Siwalik Hills in India (Lydekker, 1886). C2 and C3 each represents an upper bound of 4 Ma, derived from the capture of the Tsangpo by the Brahmaputra River, which occurred prior to about this time (Clark et al., 2004).

3. Results and discussion

The concatenated data set comprised 2658 aligned sites, including 1494 constant sites and 934 parsimony-informative

sites. The average GC content of the sequences was 0.45, and a χ^2 test at the 5% level of significance for differences in base frequencies showed that there was no base compositional heterogeneity among sequences, which is known to adversely affect phylogenetic inference (Jermiin et al., 2004).

In all phylogenetic analyses, glyptosternoids formed a monophyletic clade with robust statistical support (N4 in Fig. 2), which is consistent with the results of Peng et al. (2004) and Guo et al. (2005), providing a firm basis for testing the plausibility of the alternative biogeographic models. Within the glyptosternoid clade, *Glaridoglanis* and *Glyptosternon* appear to be basal, although their exact relationships have weak statistical support. The remaining glyptosternoids, except *Exostoma* lineages (*Oreoglanis*, *Pseudexostoma*, *Euchiloglanis*, and *Parachiloglanis*), form a well-supported internal clade. The divergence at N7 splits the congeneric species *Pareuchiloglanis kamengensis* and *Pareuchiloglanis sinensis*, with the latter pairing with *Euchiloglanis davidi*. This confirms that the traditional genus *Pareuchiloglanis* is not monophyletic, as has been previously postulated on the basis of osteological characters

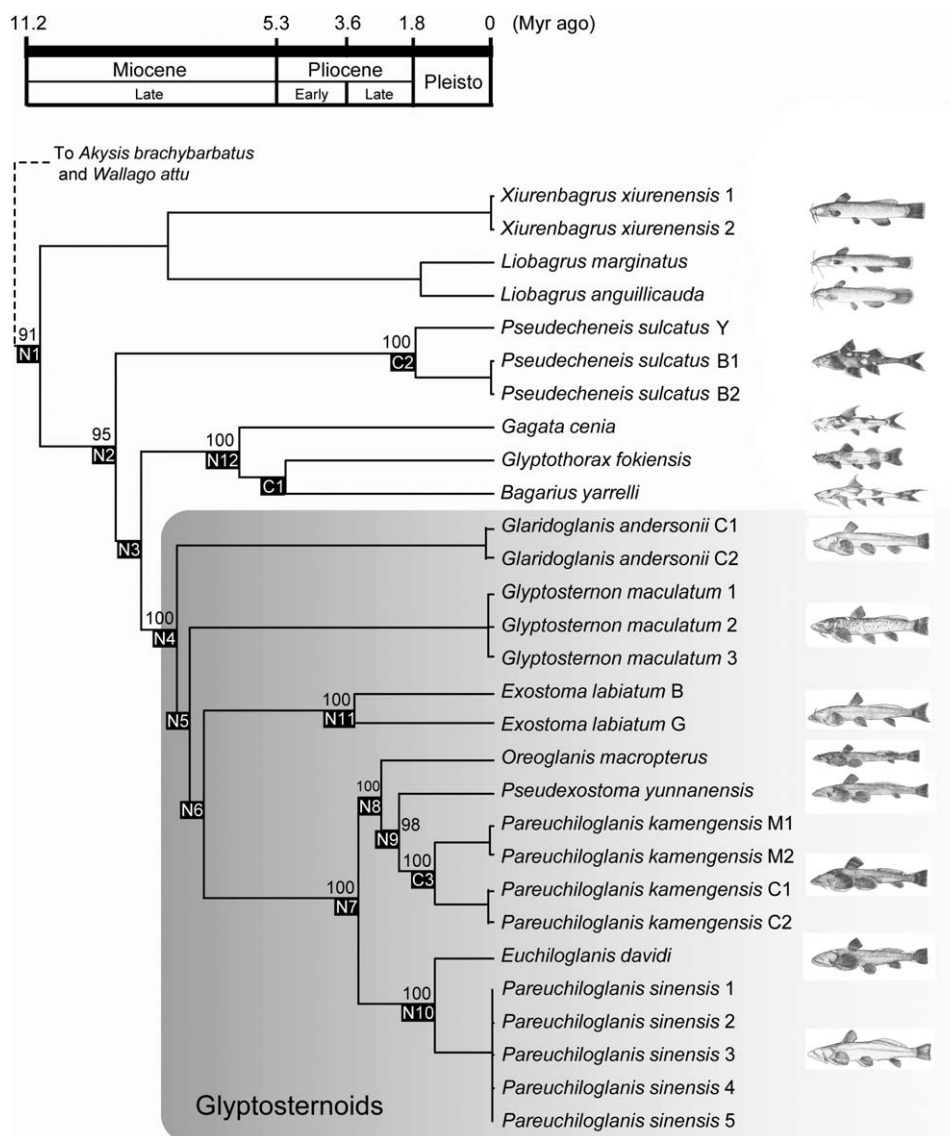


Fig. 2. Glyptosternoid tree inferred using Bayesian analysis, drawn to a time scale. C1–C3 denote nodes used for calibrating molecular date estimates. N1–N12 denote internal nodes of interest. Numbers above branches indicate posterior probabilities higher than 90%.

(He, 1996) and molecular data (Guo et al., 2005). The placement of the genus *Exostoma* is still uncertain because of comparatively low bootstrap and Bayesian posterior support, as has been previously discussed (Peng et al., 2004), and indicates a need for further investigation.

The molecular date estimates produced by the three different methods were broadly consistent (Table 2). The age of the most recent common ancestor of the glyptosternoid catfishes (N4) was estimated at about 6–8 Ma, with the next two divergences (N5 and N6) occurring shortly afterwards. A second phase of speciation is apparent, represented by nodes N7 to N9, occurring between 3.5 and 1.5 Ma ago. These molecular date estimates can be used to distinguish among competing hypotheses about the phases of uplift of the Tibetan plateau. Harrison et al. (1992) suggested that rapid uplifting and un-roofing of southern Tibet began about 20 Ma ago and that the present elevation of much of the plateau was attained by about 8 Ma ago. However, an alterna-

tive view is that the Tibetan plateau reached its maximum height before 8 Ma ago but was then lowered by extensional faulting, with a recent rapid uplift of the plateau occurring about 3.6 Ma ago accompanied by the largest glacier in the Northern Hemisphere (Cui et al., 1996; Li et al., 1996). The two hypotheses differ in a number of respects, but both recognise that the rise of the plateau likely occurred in three main steps. The molecular date estimates obtained in the present study are consistent with the second hypothesis.

The final date estimate of about 4–6 Ma for the age of C1 appears to be considerably older than the minimum bound placed on the age of this node (1.8 Ma). The calibration bound was originally specified on the basis of fossil evidence of *Bagarius yarrelli* from the sub-Himalayan Siwalik Hills, which date from the Miocene to the Pliocene (Lydekker, 1886). The molecular date estimate for C1 lies approximately on the boundary between these two epochs, which is currently dated at 5.3 Ma (van Couvering et al., 2000).

Table 2
Divergence dates estimated from molecular data using three different methods

Node ^a	Date estimate (Myr)				Penalised likelihood
	Bayesian inference (95% credibility intervals)				
	<i>Multidivtime</i>		<i>PhyBayes</i>		
C1	5.0	(4.3, 5.3)	5.3	(4.2, 6.4)	5.7
C2	1.9	(1.0, 3.1)	2.4	(1.3, 3.5)	2.0
C3	1.4	(0.8, 2.4)	1.3	(0.7, 2.0)	1.1
N1	10.9	(8.1, 14.7)	8.8	(7.7, 10.0)	10.7
N2	9.1	(7.0, 11.9)	8.0	(6.9, 9.0)	8.7
N3	8.5	(6.6, 11.0)	7.6	(6.6, 8.6)	8.2
N4	7.6	(5.8, 10.0)	6.7	(5.7, 7.6)	7.1
N5	7.3	(5.5, 9.7)	6.3	(5.4, 7.3)	6.8
N6	7.0	(5.2, 9.3)	6.1	(5.1, 7.1)	6.7
N7	3.3	(2.0, 4.9)	3.1	(2.3, 3.9)	2.5
N8	2.7	(1.6, 4.1)	2.6	(1.8, 3.5)	2.2
N9	2.3	(1.3, 3.5)	2.2	(1.4, 3.0)	1.6
N10	1.4	(0.7, 2.4)	1.7	(0.9, 2.4)	1.3
N11	3.4	(2.0, 5.1)	3.2	(2.1, 4.4)	—
N12	6.1	(5.0, 7.4)	5.9	(4.9, 7.0)	6.0

^a Node labels follow those in Fig. 2; C1–C3 are calibration nodes.

Freshwater fishes are well suited for testing hypotheses about the geological history of the Tibetan plateau. The divergence dates and the phylogenetic patterns in Fig. 2 suggest that the evolutionary history of catfishes in the Tibetan plateau is correlated with abiotic events associated with the uplift of the plateau, including the formation of isolated habitats due to rapid erosion and/or capture of rivers during the rapid uplift of the plateau. In turn, the molecular date estimates indicate that speciation periods might reflect the dates of rapid uplift. Therefore, our study has shown that molecular date estimates can be used in conjunction with geological data to investigate the history of the Tibetan plateau. Wider taxon sampling from freshwater fishes and other organisms, particularly from groups with a detailed fossil record, could provide further insights into the evolutionary history of this unique environment.

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