



## Rediscovery of the threatened Western Ghats endemic sisorid catfish *Glyptothorax poonaensis* (Teleostei: Siluriformes: Sisoridae)

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**Abstract:** *Glyptothorax poonaensis* Hora is an endemic sisorid catfish of the Western Ghats of India known only from its type locality in Mula-Mutha River, a tributary of Bhima River, at Pune. This fish has not been recorded from its type locality for more than 70 years and it was thought to be extinct. Here we report a recently discovered population of *G. poonaensis* from Indrayani River, a tributary of Bhima River. Based on 11 specimens, we have redescribed this species along with some comments on its taxonomy, length-weight relationship, feeding and breeding habits. We also performed molecular phylogeny of the fish by sequencing three mitochondrial genes encoding 16S ribosomal DNA, cytochrome *b* and cytochrome oxidase subunit I. Molecular analysis suggests that *G. poonaensis* is nested within a lineage of *Glyptothorax* species from northern and northeastern India and China. Further, our analysis reveals that southern Indian species of *Glyptothorax* do not form a monophyletic group. Molecular dating of divergence times indicates that *G. poonaensis* diverged from other northern Indian species 1.9 to 2.5 million years ago. Current knowledge suggests that the species could be found in two river basins with total extent of around 6100km<sup>2</sup>; however, the species is already suspected to be locally extinct from half of its known extent of occurrence. Furthermore, the habitat of the species may be threatened by increasing pollution, deforestation leading to siltation, halting of flow by damming, sandmining and introduced fish species. In the light of biodiversity conservation, especially in an important biodiversity hotspot like Western Ghats, such rare and endemic species needs prioritization.

**Keywords:** Conservation, *Glyptothorax poonaensis*, molecular phylogeny, northern Western Ghats.

## INTRODUCTION

The Western Ghats of India harbor a rich diversity of freshwater fish with many species endemic to this region (Shaji et al. 2000; Dahanukar et al. 2004). However, a major part of this diversity is threatened by various anthropogenic activities (Dahanukar et al. 2004), and unless serious efforts are taken to conserve natural resources, it is possible that rare and endemic species in this biodiversity hotspot may go extinct in the near future. This is especially true for stenotopic fish species like the

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ones in the genus *Glyptothorax*, which prefer clear hill streams with rapid currents. Globally, *Glyptothorax* is represented by more than 70 species (Anganthoibi & Vishwanath 2010). In the Western Ghats, *Glyptothorax* is currently represented by 10 species (Gopi 2010), namely *G. anamalaiensis* Silas, *G. annandalei* Hora, *G. davissinghi* Manimekalan & Das, *G. housei* Herre, *G. kudremukhensis* Gopi, *G. lonah* (Sykes), *G. madraspatanum* (Day), *G. malabarensis* Gopi, *G. poonaensis* Hora, and *G. trewavasae* Hora. Out of these species, *G. poonaensis* is considered to be extinct from its type locality (Kharat et al. 2003).

Hora (1938) described *G. poonaensis* as a subspecies of *G. conirostre*, from the Mula-Mutha River, a tributary of Bhima River in the northern Western Ghats, India. The description was based on five specimens, one of which was collected by A.G.L. Fraser during 1936–1937 from Kharadigaon area of Mula-Mutha River (Hora 1938; Fraser 1942). Since then at least three independent studies were carried out on the freshwater fish fauna of Mula-Mutha Rivers by Tonapi & Mulherkar (1963), Wagh & Ghate (2003; this work was actually carried out during 1992–1995) and Kharat et al. (2003) and this species has never been encountered. Studies on other nearby rivers, namely Pavana River (Chandanshive et al. 2007) and Indrayani River (Yazdani & Mahabal 1976), which are tributaries of Mula-Mutha and Bhima rivers respectively, did not record this species either. In an extensive survey of Krishna River system, Jayaram (1995) did not record this species but noted that it had been recently recorded by Ghate et al. (1992). However, Ghate et al. (1992) did not base their record on material they collected, but on records in earlier literature. *Glyptothorax poonaensis* is also mentioned in the checklist of fish from Pune District by Tilak & Tiwari (1976) but it remains unclear whether this record is based on actual collection or the existing literature. Similarly, Yadav (2003) recorded this species from the collection of Western Regional Station, Zoological Survey of India, Pune, during 1960–1995, without mentioning collection localities or dates of collection. As a result, there are no reliable records of *G. poonaensis* since its original description in 1938. This is the reason Kharat et al. (2003) considered *G. poonaensis* to be possibly extinct in the Mula-Mutha River after a detailed survey of the river drainage.

In a recent survey of Indrayani River, also a

tributary of Bhima River (and very close to the Mula-Mutha River of Pune; approximately 18km by land but around 120km river stretch from the type locality of the fish) we discovered a population of *G. poonaensis*. In this paper we redescribe the species with further details on its morphometry, molecular phylogeny, current distribution, length-weight relationship, feeding habits, breeding habit and threats to the species. This data will be helpful while designing and implementing conservation strategies.

## METHODS

### Study area and specimen collection

The Indrayani River is a tributary of Bhima River, which is itself a major tributary of the Krishna River system. In our study on the fish diversity of Indrayani River, specimens of *Glyptothorax* were collected at Markal (18.671°N, & 73.981°E) from June to August 2010 from the local fishermen. The specimens were preserved in 4% formaldehyde and were identified based on available literature (Hora 1938; Silas 1951; Talwar & Jhingran 1991; Jayaram 2010). Four specimens from our study are deposited in the collection of Wildlife Information Liaison Development, Coimbatore under the accession numbers WILD-11-PIS-004, WILD-11-PIS-005, WILD-11-PIS-006 and WILD-11-PIS-018. Three specimens from our collection are deposited in the Zoological Survey of India, Western Regional Centre, Akurdi, Pune under the accession numbers ZSI Pune P/2431, P/2432 and P/2433.

### Biological and morphological data collection and analysis

Morphometric and meristic data were collected following Vishwanath & Linthoingambi (2005), Ng & Kottelat (2008) and Jayaram (2010). Wherever there was discrepancy in the definition of a morphometric character, for example body depth at anus (Ng & Kottelat 2008) versus body depth at dorsal origin (Jayaram 2010), we took both the measurements and recorded them appropriately. In all 40 morphometric characters and four meristic characters were considered. Morphometry was performed using dial calipers with a least count of 0.0254mm. We also extracted morphometric data for *G. poonaensis*, *G. conirostre*, *G. lonah* and *G. trewavasae* from Hora (1938) for

comparison. To avoid personal measurement biases while comparing Hora's (1938) data and ours we performed cluster analysis based on the mean values of size adjusted morphometric data. We calculated Euclidian distances and we used Ward's method of clustering. Weight of each individual was measured on an electronic weighing balance with a least count of 0.01g. We plotted log-log plot of length and weight of the fish to determine the exponent of the length-weight relationship given by the equation  $W = aL^b$ , where 'W' is the weight, 'a' is the normalization constant, 'L' is the length and 'b' is the scaling exponent. The null hypothesis that 'b=3' was tested by comparing the 95% confidence interval of calculated value of 'b' (Quinn II & Deriso 1999, pp.131). We dissected three specimens in our collection to extract their gut contents and analyzed their feeding habits.

#### DNA isolation and molecular phylogeny

Muscle tissues were harvested from two fresh specimens (WILD-11-PIS-018 and ZSI Pune P/2431) and were preserved in absolute ethanol. The tissue was digested at 50°C for two hours using the STE buffer (0.1M NaCl, 0.05 M Tris-HCl, 0.01M EDTA, 1%SDS) with 15µl proteinase K (20mg/ml) per 500µl of STE buffer. DNA was extracted using conventional phenol-chloroform method and re-suspended in nuclease free water. Polymerase chain reaction was performed to amplify three fragments of mitochondrial genes namely cytochrome b (cyt-b), cytochrome oxidase subunit I (cox1) and 16S ribosomal DNA (16S rDNA). Primers used for the amplification are given in the Table 1. PCR was performed in a 50µl reaction volume containing 5µl of template DNA (~200ng), 5µl of 10x reaction buffer (100mM Tris pH 9.0, 500mM KCl, 15mM MgCl<sub>2</sub>, 0.1% gelatin), 2µl of 25mM MgCl<sub>2</sub>, 2µl of 10mM dNTPs, 1µl of each primer, 1µl

taq polymerase and 33µl distilled water. The genes were amplified using gene-specific primers. For 16S rDNA thermal profile was 5min at 95°C, and 40 cycles of 30 seconds at 94°C, 30 seconds at 61°C and 2min at 72°C followed by extension of 10min at 72°C. For cyt-b and cox1 thermal profile was 5min at 95°C, and 40 cycles of 30 seconds at 94°C, 30 seconds at 51°C and 2min at 72°C, followed by extension of 10min at 72°C. Amplified DNA fragments were purified using the 'Promega Wizard Gel and PCR clean up' system and sequenced. The purified PCR products were sequenced using ABI prism 3730 sequencer (Applied Biosystems, USA) and big dye terminator sequencing kit (ABI Prism, USA). Sequences were analyzed by BLAST tool (Altschul et al. 1990). These sequences have been deposited in Genbank.

We retrieved additional sequences of other species of *Glyptothorax* from NCBI GenBank (<http://www.ncbi.nlm.nih.gov/>). We used *Gagata cenia* and *Bagarius yarrelli* as out-groups following Peng et al. (2006). All the sequences used for the analysis, along with their GenBank accession number, are given in Table 2. Our total data matrix encompasses fragments of three mitochondrial genes namely 16S rDNA, cyt-b and cox1 with a total sequence length of 2120 bp after alignment. Sequences were aligned using ClustalW (Thompson et al. 1994). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al. 2011). Best fit model for nucleotide substitution was selected based on minimum Akaike Information Criterion (AIC) value (Posada & Crandall 2001). We constructed phylogenetic trees based on maximum parsimony, minimum evolution, neighbour joining and maximum likelihood. Reliability of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations. Based on the calibration points available

**Table 1. Primer sequences used in this study**

Gene amplified	Primer	Sequence (5' → 3')	Tm	Approximate size of amplification (bp)	Reference
cyt-b	L14724 (F)	GACTTGAAAAACCACCGTTG	50	1118	Chen et al. (2007)
	H15915 (R)	CTCCGATCTCCGGATTACAAGAC	57		
cox1	FishF1	TCAACCAACCACAAAGACATTGGCAC	58	655	Sharina & Kartavtsev (2010)
	FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	58		
16S rDNA	16Sar-F	CGCCTGTTTATCAAAAACAT	46	573	Ivanova et al. (2007)
	16Sbr-R	CCGGTCTGAACTCAGATCACGT	57		

**Table 2. GenBank details of gene sequences used for phylogenetic analysis**

Species	GenBank accession numbers			Voucher specimen number
	16S rDNA	Cytochrome b	Cytochrome oxidase-1	
<i>Glyptothorax brevipinnis</i>	FJ357193	FJ208934	FJ208927	NBFGR:GB1
<i>Glyptothorax cavia</i>	AY445906	AF477830*	DQ514343	IHB200106243 (for 16S rDNA) UMMZ 247440 (for Co-1)
<i>Glyptothorax dakpathari</i>	FJ357197	EU637440	EU637832	NBFGR:GP3
<i>Glyptothorax davissinghi</i>	FJ357212	FJ423582	FJ347795	NBFGR:GD8318G
<i>Glyptothorax fokiensis</i>	AY574359	AF416884*	DQ514346	IHB3811 (for 16S rDNA) UMMZ 245065 (for Co-1)
<i>Glyptothorax garhwali</i>	FJ357186	EU637428	EU637803	NBFGR:GG1
<i>Glyptothorax granulus</i>	FJ357181	FJ349168	FJ347865	NBFGR:GR8413X
<i>Glyptothorax ngapang</i>	FJ357173	FJ349120	FJ347817	NBFGR:GN8412K
<i>Glyptothorax poonaensis</i>	JN092395	JN092396	JN092397	WILD-11-PIS-018
<i>Glyptothorax poonaensis</i>	HQ833462	HQ833463	HQ833464	ZSI, Pune, P/2431
<i>Glyptothorax sinensis</i>	AY574357	AY601764	DQ514360	IHB0305147 (for 16S rDNA) IHB0305147 (for Cyt-b) UMMZ 246438 (for Co-1)
<i>Glyptothorax ventrolineatus</i>	FJ357203	FJ349177	FJ347797	NBFGR:GV8417A
<i>Gagata cenia</i>	AY445905	AF499599*	DQ846700	IHB9907005 (for 16S rDNA) UMMZ 247439 (for Co-1)

\* Voucher specimen not specified

on the molecular dating of *Gagata cenia* and *Bagarius yarrelli* (Peng et al. 2006) we performed molecular dating using maximum likelihood analysis based on cyt-b gene sequence. A molecular clock test was performed to find out whether the substitution rates were uniform (Tamura et al. 2011).

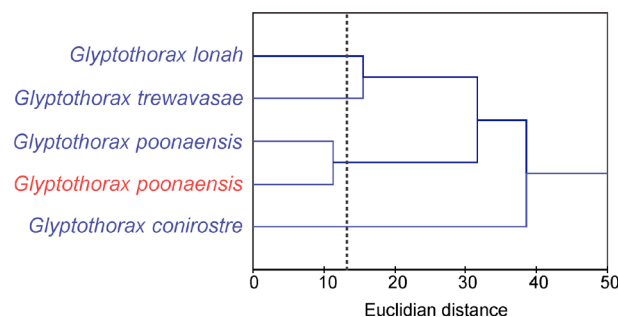
## RESULTS AND DISCUSSION

In spite of several extensive surveys in the Mula-Mutha River and other rivers in the vicinity of Pune (Tonapi & Mulherkar 1963; Yazdani & Mahabal 1976; Kharat et al. 2003; Wagh & Ghate 2003; Chandanshive et al. 2007), *G. poonaensis* has not been recorded after its description by Hora (1938). The only other records of the fish by Tilak & Tiwari (1976) and Yadav (2003) could be based only on existing literature (see Introduction). As a result our report of *G. poonaensis* is the first verifiable record since its original description.

### Taxonomic comments

We compared the specimens in our collection with the original description in Hora (1938) and confirmed their conspecificity. We further confirmed the identity of *G. poonaensis* by comparing our morphological

data with the data for *G. poonaensis*, *G. conirostre*, *G. lonah* and *G. trewavasae* from Hora (1938). Size adjusted morphometric data from Hora (1938) and our specimens (Table 3) agree closely, further verifying their conspecificity. The only disagreement between our data and that in Hora (1938) is the length of caudal peduncle. Our measurements (19.3–22.7 % SL) are higher than those reported by Hora (16.6–17.5 % SL), however, we cannot comment on the reasons behind this difference because of the small sample sizes in both cases. The dendrogram (Fig. 1) based on the morphometrical comparisons (Table 3) suggests that



**Figure 1. Comparison of morphometric data of present collection with Hora's (1938) data. Dendrogram is plotted using Euclidian distances and Ward's method. (Species names shown in blue are based on Hora's (1938) data, while red corresponds to present study). Dashed line is the significance line.**

**Table 3. Comparison among the morphometry of *Glyptothorax* given by Hora (1938) and present study. All characters are expressed as average of percentage of standard length with their standard deviations in the parenthesis.**

Characters % of standard length	Data from Hora (1938)				Present study
	<i>G. poonaensis</i>	<i>G. conirostre</i>	<i>G. lonah</i>	<i>G. trewavasae</i>	<i>G. poonaensis</i>
Length of head	23.6 (0.36)	22.7 (0.73)	23.8 (0.66)	25.7 (0.68)	23.8 (1.40)
Width of head	20.2 (1.44)	16.7 (0.22)	20.1 (0.33)	20.8 (0.43)	19.1 (1.32)
Height of head	13.0 (1.03)	12.1 (0.05)	14.2 (0.52)	12.8 (1.16)	13.3 (1.08)
Length of snout	11.5 (1.08)	11.6 (0.52)	11.8 (0.86)	13.5 (1.51)	12.3 (0.36)
Inter-orbital width	6.4 (0.32)	5.4 (0.97)	8.0 (0.67)	7.4 (0.52)	7.1 (0.59)
Depth of body	15.5 (1.83)	15.1 (1.17)	18.2 (1.04)	15.5 (1.42)	17.1 (1.12)
Height of dorsal fin	16.8 (0.97)	19.9 (0.36)	18.5 (1.41)	19.4 (2.11)	16.8 (0.86)
Length of pectoral fin	21.6 (1.30)	24.2 (0.10)	23.5 (0.57)	22.1 (3.37)	20.6 (1.40)
Length of ventral fin	15.8 (1.48)	17.8 (1.39)	19.0 (0.58)	16.9 (0.97)	14.8 (0.72)
Longest ray of anal fin	14.2 (1.91)	18.9 (0.26)	18.5 (1.40)	17.5 (2.68)	15.8 (1.28)
Length of dorsal spine	12.5 (0.16)	14.2 (0.86)	13.1 (0.95)	13.4 (0.86)	12.4 (0.63)
Length of caudal peduncle	17.1 (0.46)	21.3 (1.25)	17.6 (1.06)	17.8 (0.83)	20.7 (0.88)
Least height of caudal peduncle	8.2 (0.33)	9.1 (0.02)	11.1 (0.74)	8.6 (1.52)	8.5 (1.35)

not only our specimens are similar to *G. poonaensis* sensu stricto but *G. poonaensis* is significantly different from its closely related northern Indian species *G. conirostre* and other species, namely *G. lonah* and *G. trewavasae*, described from the northern Western Ghats of India.

*Glyptothorax poonaensis* was originally described as a valid subspecies of *G. conirostre*. Menon (1999) elevated its status to the species level without giving any rationale and the trend was followed by Thomson & Page (2006). Jayaram (2009, 2010) however still considers the species as a valid subspecies of *G. conirostre*, while, Ferraris (2007) has considered the species as *species inquirendae* owing to the fact that the species has either been treated as valid or as a synonym of *G. conirostre*. Our analysis based on the data available on *G. conirostre* in Hora (1938) and comparison of our material of *G. poonaensis* with the original description of *G. conirostre* given by Steindachner (1867) indicates that *G. poonaensis* is a valid species markedly distinct from *G. conirostre*. Comparison of standardized characters given in (Table 3) suggests that *G. conirostre* is different from *G. poonaensis* in the head width and lengths of different fins. As compared to *Glyptothorax conirostre*, *G. poonaensis* has lesser height of dorsal fin (15.1–18.1 % SL vs. 19.6–20.1 % SL), length of pectoral fin (18.6–22.8 % SL vs. 24.2–24.3 % SL), length of ventral fin (13.7–15.8 % SL vs. 16.8–18.8 % SL), length of

longest anal fin ray (13.8–18.4 % SL vs. 18.7–19.1 % SL) and length of dorsal spine (11.1–13.4 % SL vs. 13.6–14.8 % SL). However, as compared to *G. conirostre*, *G. poonaensis* has larger head width (17.0–21.2 % SL vs. 16.5–16.8 % SL) and inter orbital width (6.2–8.3 % SL vs. 4.7–6.0 % SL). Jayaram (2009) has separated *G. poonaensis* from *G. conirostre* based on having broader head, shorter dorsal fin and posterior insertion of pelvic fins. We therefore suggest that *G. poonaensis* should be considered as a valid species distinct from *G. conirostre*.

*Glyptothorax poonaensis* can be differentiated from nine other species of *Glyptothorax* known from the Western Ghats using a combination of characters (Hora 1938; Silas 1951; Talwar & Jhingran 1991; Jayaram 2009; Gopi 2010). *Glyptothorax poonaensis* differs from other species known from the Western Ghats, namely *G. anamalaiensis*, *G. annandalei*, *G. davissinghi*, *G. kudremukhensis*, *G. lonah*, *G. trewavasae* and *G. madraspatanum*, in having smooth skin as oppose to tuberculated or granulated skins in all other species. Apart from the difference in the skin character, *G. poonaensis* can be easily differentiated from *G. anamalaiensis* in lacking (vs. having) three broad white bands on the body. *Glyptothorax annandalei* and *G. davissinghi* can be separated from *G. poonaensis* in having plaited (vs. smooth) ventral surfaces of the paired fins. *Glyptothorax poonaensis* can be differentiated from *G. annandalei*,

*G. davissinghi*, *G. lonah*, *G. trewavasae* and *G. madraspatanum* in having poorly developed (vs. well developed) adhesive apparatus. Here, poorly developed adhesive thoracic apparatus can be defined as consisting of ridges of skin with only 16 prominent striae and only anterior striae well defined while posterior striae indistinct, as oppose to well developed adhesive thoracic apparatus with more than 20 striae and both anterior and posterior striae distinctly visible. Other *Glyptothorax* species found in the Western Ghats, which have smooth skin, are *G. housei* and *G.*

*malabarensis*. *Glyptothorax poonaensis* differs from *G. housei* by a poorly developed (vs. well developed) adhesive thoracic apparatus, smooth (vs. plaited) ventral surfaces of the paired fins, shorter (vs. longer) maxillary and nasal barbels and dorsal fin origin nearer to adipose fin than to tip of snout (vs. nearer to tip of snout than adipose fin base). *Glyptothorax poonaensis* differs from *G. malabarensis* in having the thoracic adhesive apparatus poorly developed and longer than broad (vs. moderately developed, pentagonal in shape and as long as broad).



Image 1. Details of *Glyptothorax poonaensis* (WILD-11-PIS-004). a - Lateral; b - dorsal; c - ventral view.

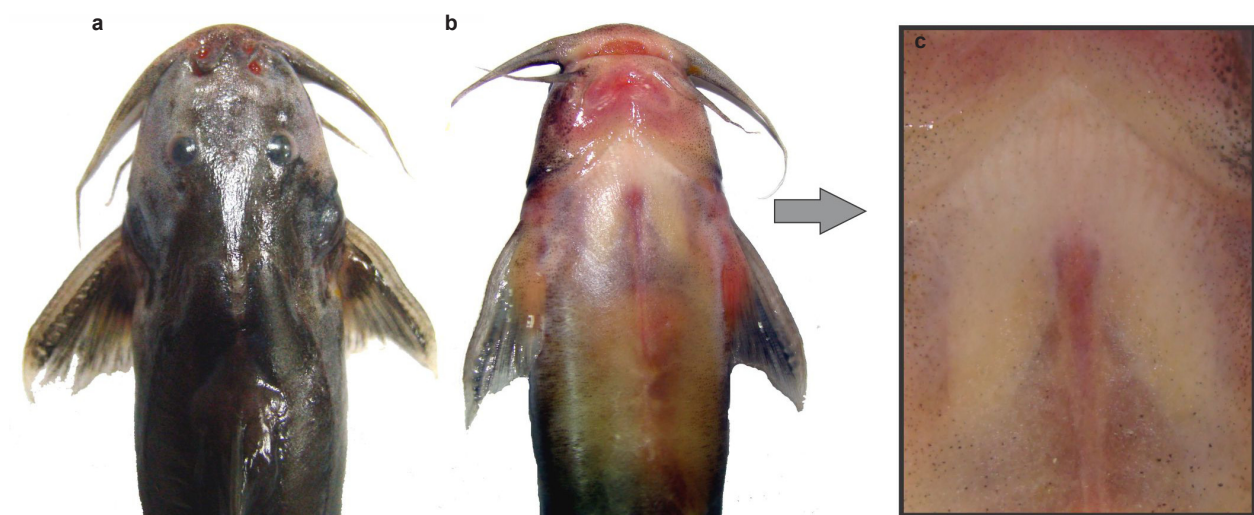


Image 2. Details of the head and adhesive thoracic apparatus of *Glyptothorax poonaensis* (ZSI Pune P/2431)  
a - Dorsal profile of head; b - ventral profile of head; c - details of adhesive thoracic apparatus.



Image 3. (a) Smooth (WILD-11-PIS-004), and (b) wrinkled (WILD-11-PIS-018) skin of *Glyptothorax poonaensis* and its comparison with (c) tuberculated skin of *G. ionah* (unregistered specimen collected from Koyna River).

### Redescription of *Glyptothorax poonaensis*

Detailed morphometry of *G. poonaensis* is given in Table 4 and details of the body structure, head structure and details of skin are presented in Images 1, 2 and 3 respectively. The redescription is based on the freshly collected specimens as indicated in Table 4. The specimens deposited in WILD match exactly in morphology the types (4 specimens bearing the registration number F12126/1) deposited at the Zoological Survey of India, Kolkata.

Head depressed, body subcylindrical. Dorsal profile rises evenly from tip of snout to origin of dorsal fin then slopes gently ventrally from origin of dorsal fin to end of caudal peduncle. Ventral profile flat from snout tip to anal fin base, then slopes gently dorsally from anal fin base to end of caudal peduncle. Anus and urogenital openings located at vertical through middle of adpressed pelvic fin. Skin smooth on body and head but could be wrinkled in some specimens occurring

either as a preservation artifact or molting (Image 3). Lateral line complete, mid-lateral in position.

Head depressed and broad. Snout prominent. Anterior and posterior nares large and separated only by base of nasal barbels. Gill opening broad, extending from immediately ventral to post-temporal to isthmus. Bony elements of dorsal surface of head covered with thick, smooth skin. Occipital process does not reach base of dorsal fin. On either side of supraoccipital process, supracleithrum forms two finger like projections separated by large interspace as shown by Hora (1938: fig. 1c). Eye ovoid, located entirely on dorsal half of head, its horizontal axis longest, its diameter 9.8-13.5% HL.

Barbels four pairs, maxillary barbel long and slender extending to middle of pectoral fin base. Nasal barbels slender, barely reaching eye. Inner mandibular barbel origin close to midline extending up to gill opening on ventral surface. Outer mandibular barbel

**Table 4. Raw data of morphometric (mm) and meristic characters of *Glyptothorax poonaensis***

Character	GP1 <sup>a, b</sup>	GP2 <sup>a, c</sup>	GP3	GP4 <sup>c</sup>	GP5 <sup>b</sup>	GP6	GP7 <sup>c</sup>	GP8	GP9 <sup>b</sup>	GP10 <sup>b</sup>	GP11
Total length	128.14	112.98	111.13	110.08	108.38	144.55	124.46	111.30	108.97	109.27	104.60
Standard length	105.56	91.95	90.96	91.67	89.71	120.50	102.24	94.23	89.46	90.55	87.12
Length of caudal	24.38	19.56	19.05	19.38	19.76	24.87	21.95	19.00	21.46	19.81	17.68
Pedorsal length	37.34	31.12	31.50	32.84	32.69	40.51	35.94	35.97	31.80	31.88	30.45
Pre anal length	63.27	54.79	53.34	52.98	54.97	70.99	61.75	56.87	52.73	55.85	52.91
Prepelvic length	53.01	44.60	43.38	43.33	44.04	57.56	49.66	48.90	43.36	46.28	42.34
Pre pectoral length	18.75	17.27	16.64	17.40	16.81	20.88	17.50	16.89	16.51	17.07	16.71
Length of head	25.96	19.00	20.70	22.20	22.58	28.50	22.61	23.04	21.89	22.61	21.59
Depth of head at occiput	11.56	11.38	11.56	11.99	12.40	17.15	14.10	12.27	13.18	13.08	11.56
Depth of head at eye	9.65	8.81	8.48	9.19	9.45	12.78	10.36	8.59	9.35	8.76	8.46
Width of head	17.96	15.80	16.64	16.64	18.14	25.53	20.02	18.92	17.17	17.37	17.53
Width of head near nares	11.13	10.16	11.38	10.77	12.19	15.60	11.99	10.80	10.72	10.16	9.86
Length of snout	12.58	11.45	11.36	11.42	11.26	13.82	12.3	11.64	11.24	11.33	11.05
Eye diameter	2.69	2.57	2.54	2.54	2.57	2.79	2.67	2.41	2.59	2.92	2.69
Interorbital width	6.63	6.48	6.81	6.35	5.59	8.59	7.01	7.11	7.44	6.60	5.92
Internarial space	2.67	2.57	3.00	3.05	2.90	4.17	3.51	3.43	3.71	3.81	3.18
Gape width	10.34	8.76	8.69	8.53	9.63	11.07	8.74	9.91	8.86	9.27	8.38
Nasal barbel length	8.92	6.48	6.76	6.58	7.21	9.14	9.14	6.96	7.95	8.08	8.20
Maxillary barbel length	23.57	19.69	21.62	18.39	20.47	30.78	27.05	22.28	21.34	19.10	18.67
Innermandibular barbel length	10.26	9.63	7.34	7.62	7.92	9.02	7.24	8.36	8.79	7.75	8.38
Outer mandibular barbel length	15.57	13.67	11.89	12.50	12.75	15.93	13.16	12.70	13.34	11.53	12.01
Depth of body at dorsal origin	18.14	15.80	16.51	14.27	16.74	22.38	18.26	15.72	15.04	14.68	13.41
Depth of body at anus	15.24	13.82	14.12	13.08	14.43	21.03	17.22	13.13	13.67	13.26	12.83
Width of body at dorsal origin	16.94	15.54	14.78	14.76	17.30	19.46	16.05	17.53	15.37	16.38	14.88
Width of body at anal origin	11.23	12.24	10.72	11.07	12.32	14.83	12.45	10.97	11.51	11.13	10.21
Height of dorsal fin	17.58	15.11	13.69	15.29	15.77	21.03	18.47	14.86	15.24	14.86	15.04
Length of dorsal fin base	12.24	11.05	11.48	11.02	10.95	13.26	10.21	10.54	11.25	10.39	10.41
Dorsal spine length	11.76	11.68	10.87	11.33	11.40	15.88	12.70	11.18	11.00	11.53	11.68
Length of adipose fin base	10.97	10.36	10.36	9.68	9.53	13.54	9.93	9.19	10.54	9.65	10.67
Post adipose distance	20.98	19.38	17.83	17.91	17.40	23.11	21.21	18.82	17.78	17.58	16.89
Dorsal to adipose distance	25.02	21.84	21.39	22.73	22.15	32.64	29.46	23.37	21.26	24.26	20.88
Length of pectoral fin	21.46	19.46	16.89	17.58	19.33	27.13	23.32	17.65	18.69	18.85	17.73
Length of pectoral spine	15.09	15.11	12.45	14.61	14.35	22.86	20.83	14.35	13.54	13.77	14.20
Length of ventral fin	16.66	13.79	12.47	13.16	13.16	18.62	16.13	12.95	13.41	13.46	12.45
Length of anal fin base	11.40	9.91	8.33	9.65	7.90	12.50	11.73	10.62	10.03	11.28	9.83
Length of largest anal fin ray	16.66	15.11	13.41	14.02	13.61	16.64	16.99	14.10	15.47	16.64	13.54
Length of caudal peduncle	22.00	18.97	19.33	18.42	18.67	27.31	21.44	18.21	18.75	17.91	17.53
Least height of caudal peduncle	8.33	7.11	7.01	7.44	6.88	13.34	11.53	7.16	7.11	7.16	7.75
Length of adhesive apparatus	14.12	13.67	13.56	12.65	12.60	16.97	15.72	11.94	15.06	14.10	12.90
Width of adhesive apparatus	13.34	12.70	11.99	10.36	11.46	11.56	12.34	10.52	11.94	10.67	10.72
Dorsal fin rays	16	16	16	16	16	15	16	16	16	16	16
Pectoral fin rays	17	18	18	18	19	19	19	18	18	18	18
Ventral fin rays	15	15	15	15	15	15	15	15	15	15	15
Anal fin rays	11	11	11	11	11	11	11	11	11	11	11

<sup>a</sup> - specimens used for DNA isolation; <sup>b</sup> - deposited at Wildlife Information Liaison Development, Coimbatore, Accession numbers: GP1 = WILD-11-PIS-018, GP5 = WILD-11-PIS-004, GP9 = WILD-11-PIS-005, GP10 = WILD-11-PIS-006; <sup>c</sup> - deposited at Zoological Survey of India, Western Regional Centre, Pune, Accession numbers: GP2 = P/2431, GP4 = P/2432, GP7 = P/2433



originating posteriolateral of inner mandibular barbel extending to origin of pectoral fin. Mouth inferior, premaxillary tooth band partially exposed when mouth is closed. Oral teeth small and villiform in irregular rows, premaxillary teeth in single broad semilunate band. Dentary teeth in two narrow crescentic bands separated at midline.

Dorsal fin located above anterior third of body with I,5 (N=1) or I,6 (N=10) rays; fin margin convex; spine short and gently curved. Adipose fin with anterior margin concave. Caudal fin strongly forked with lower lobes slightly longer than upper lobes. Procurent rays symmetrical, extending slightly anterior on fin base. Anal fin base ventral to adipose fin origin. Anal fin with concave anterior margin and straight posterior margin with ii,8 (N=8) or ii,9 (N=3) rays. Pelvic fin origin slightly behind the posterior end of dorsal fin base. Pelvic fin with slightly convex margin and i,5 rays. Tip of adpressed fin not reaching anal fin origin but covers anus and urogenital area. Pectoral fin with I,7 (N=1) or I,8 (N=7) or I,9 (N=3) fin rays posterior fin margin slightly convex. Anterior spine margin smooth, posterior margin with 17–21 serrations.

Thoracic adhesive apparatus present, weakly developed, forming a narrow band and somewhat V shaped appearance (Image 2c), with median depression present on posterior half and extending from isthmus to level of middle of pectoral fin. Median ridges oriented longitudinally, ridges uninterrupted.

In 4% formaldehyde dorsal and lateral surfaces of head grayish with yellow tinge. A dark gray patch on the dorsal profile extending from occipital process to posterior base of adipose fin. Ventral surface yellow to pale yellow. Dorsal fin gray with white band in middle, pectoral, ventral and anal fins with yellow base and gray tips. Caudal fin with black or dark gray base followed by gray tips. Head and body studded with randomly-distributed minute black dots. On the lateral surfaces of the body gray with yellow tinge sometimes give rise to brownish coloration.

### Molecular phylogeny

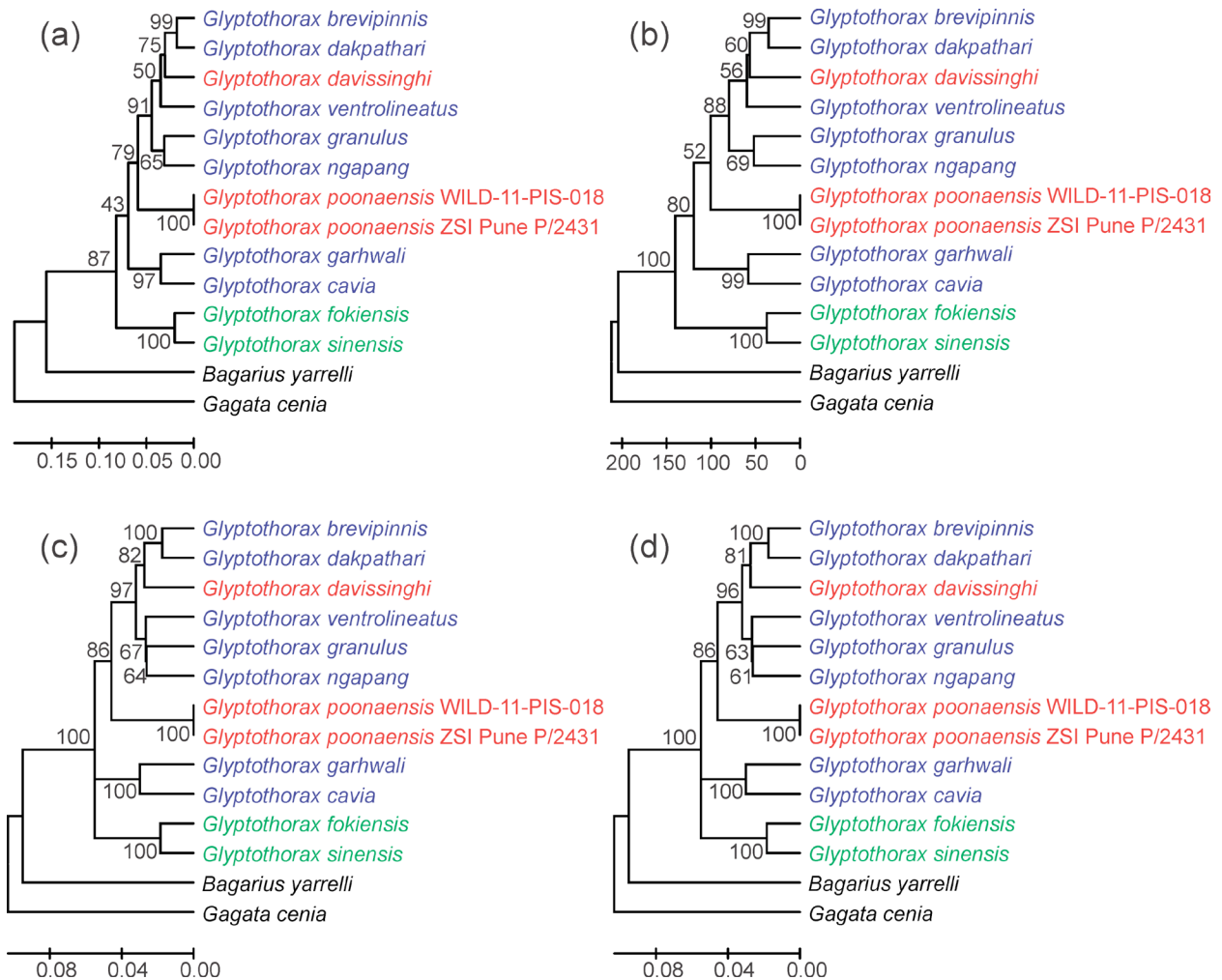
ModelTest in MEGA 5 (Tamura et al. 2011) suggested that General Time Reversible + Gamma + Proportion Invariant (GTR+G+I) model was the best fit model for nucleotide substitution for our data and thus it was applied to generate phylogenetic hypothesis. The results for phylogenetic analysis

based on maximum likelihood, maximum parsimony, minimum evolution and neighbor joining are shown in Fig. 2. The phylogeny based on a combined analysis of all three data partitions (16S rDNA, cyt-b and cox1) suggests that *G. poonaensis* is nested within a clade consisting of species from the northern part of the Indian subcontinent and China (Fig. 2). Interestingly, *G. poonaensis* and *G. davissinghi*, both Western Ghats species, were separated by a larger distance suggesting that southern Indian *Glyptothorax* do not form a monophyletic group.

Molecular dating of divergence times between *G. poonaensis* and other northern Indian species suggests that *G. poonaensis* diverged from other northern Indian species between 1.9 to 2.5 million years ago (Fig. 3). We used only cyt-b gene for molecular dating because (Peng et al. 2006) have considered cyt-b gene for their analysis and we are using same reference for the calibration. Further, our total data matrix consisting of 16S rDNA, cyt-b and cox1 had non uniform substitution rates. Also, we could not use 16S rDNA and cox1 genes individually as their sequence length was small for statistical analysis. However, for cyt-b the sequence length was adequate for analysis and the null hypothesis of equal evolutionary rate throughout the tree was not rejected ( $p=0.2065$ ) making the molecular dating more reliable.

### Distribution and population status

The collection locality of one paratype of *G. poonaensis* is Kharadigaon (18.545°N & 73.949°E), Pune (Hora 1938; Fraser 1942), while the type locality (where three paratypes were also collected) is more vague, being merely given as “near Poona [=Pune]”. We collected the species from the Village Markal (18.671°N & 73.981°E) situated on the right bank of Indrayani River, Pune. The two collection sites are numbered 1 and 2 respectively in Fig. 4 and the associated river basins are highlighted in blue. The total area drained by the two river basins is 6105.4km<sup>2</sup>. However, it is suspected that the species could be locally extinct from the Mula-Mutha River (Kharat et al. 2003), indicating that the current extent of occurrence (IUCN 2001) of this fish could be reduced by at least half. We lack estimates of the population size of this species, but it is possible that it is relatively rare. This is based on the fact that in the extensive survey of Mula-Mutha River by Fraser (1942) only one specimen was collected.



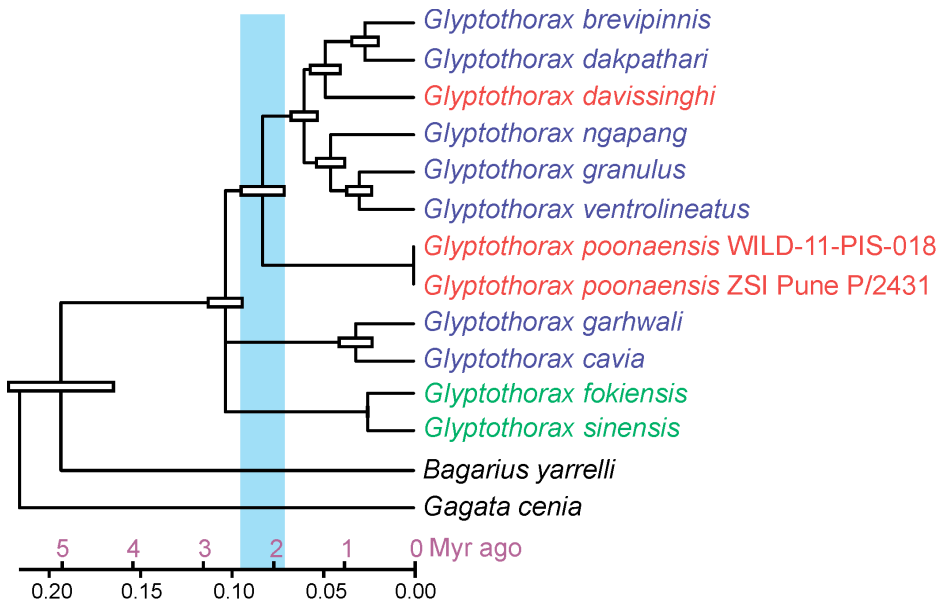
**Figure 2. Phylogenetic position of *Glyptothorax poonaensis*.** Phylogenetic tree constructed using (a) maximum likelihood, (b) maximum parsimony, (c) minimum evolution and (d) neighbor joining analysis using 2120 base pairs of partial 16S rDNA, cyt-b and cox1 gene sequences. Values at each node are Bootstrap values for 1000 iterations. *Gagata cenia* and *Bagarius yarrelli* are used as out groups. Species names in blue indicate northern Indian and northeastern Indian species, species names in green indicate Chinese species and species name in red are Western Ghats species.

Further, previous study on Indrayani River (Yazdani & Mahabal 1976) failed to collect this species. Our discussions with the fishermen revealed that this species, locally called as Patthar-chatu, is found only during summer and early rainy season when the water level is low and even during this period the fish is very rare. However, since directed fishing efforts for this particular species are not done, we do not have exact estimates for the species abundance.

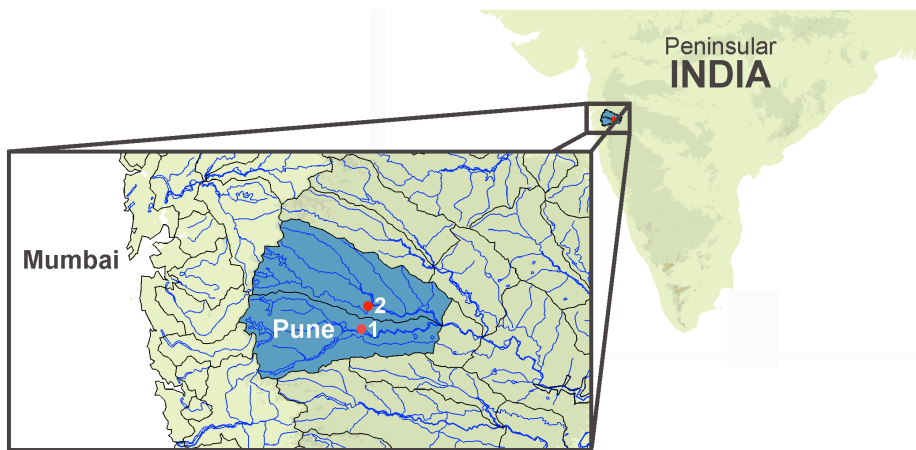
### Biology

The length weight relation of unsexed *G. poonaensis* can be described by the equation  $W=0.0087L^{3.2436}$  (Fig. 5,  $R^2=0.933$ ,  $p < 0.001$ ). Since, the 95% confidence interval of scaling exponent was in the range 2.5882-

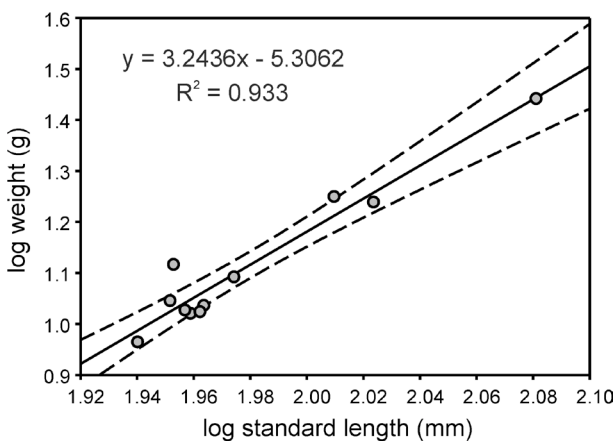
3.8989, the null hypothesis, stated as the found value of the exponent is not significantly different from the predicted value of 3 by isometry, could not be rejected. Thus the fish grows isometrically. Gut content of three specimens of *G. poonaensis* suggested that the fish feeds on benthic macroinvertebrates such as freshwater prawns (Image 4A), maxillopod crustacean (Branchiura) (Image 4B) and Odonata nymph (Image 4C). Our collection of gravid females suggests that this species probably breeds during June to August (the monsoon season). Similar observations are also made on other species of *Glyptothorax* (Dobriyal & Singh 1993; Nath 1994; Kaul 1994). Based on the fact that *Glyptothorax* are known to migrate downstream for breeding (Kaul 1994), we suspect that Markal could



**Figure 3. Molecular dating of *Glyptothorax poonaensis*** based on maximum likelihood analysis of *cyt-b* gene and the molecular calibrations available for divergence time between *Gagata cenia* and *Bagarius yarrelli* and between *Bagarius yarrelli* and *Glyptothorax fokiensis* (Peng et al. 2006). White bars on each node represent the 95% confidence interval of divergence time. Color code for species names as per Figure 2.



**Figure 4. Distribution of *Glyptothorax poonaensis*.** Species is known only from two localities: (1) Kharadigaon (18.545°N & 73.949°E) (2) Markal (18.671°N & 73.981°E).



**Figure 5. Length weight relationship of *Glyptothorax poonaensis*.** Dashed lines indicate 95% confidence interval.

be the breeding ground for *G. poonaensis*. This is because Markal is located on the main river and there are no hill streams in its immediate vicinity. Further, according to the local fishermen, the species is found in Markal area only during June to August, which is thought to be the breeding period of the fish.

**Threats and conservation measures**

There are several threats to *G. poonaensis* in its known range. Important threats to hill stream species like *G. poonaensis* include alteration of hydrological regimes because of damming, increasing pollution, deforestation leading to siltation, and introduced fish species. Dams cut the flow of water and lessen the speed of water creating semi-lacustrine conditions. These conditions may be highly disliked by hill



**Image 4. Food items from the gut of *Glyptothorax poonaensis* - (a) freshwater prawn, (b) maxillopod crustacean (*Branchiura*) and (c) *Odonata* nymph.**

stream fishes like *Glyptothorax*, which are specialized for living in torrent streams (Hora 1930). Also, *Glyptothorax* utilize gravel bed areas for spawning which are lost in rivers immediately below the dams (Kaul 1994; Nath 1994). Further, impeded water flow (both upstream and downstream of the dam) can lead to eutrophication and the creation of oxygen-poor habitats. As species of genus *Glyptothorax* require large amounts of oxygen in water (Hora 1930) such habitat alterations are unsuitable for the species. Similar habitat alterations can also result from urbanization leading to organic pollution in rivers. Such changes in the rivers near the study area and their possible effects on the fish fauna are already documented (Kharat et al. 2003; Wagh & Ghate 2003). As stated before, we suspect that Indrayani River at Markal could be the breeding ground for *G. poonaensis* as *Glyptothorax* species are known to migrate downstream for breeding (Kaul 1994). If this is true then pollution in this area, which is an ongoing threat, is of major concern. An ongoing threat in terms of deforestation, leading to siltation, can also affect the breeding grounds of the fish. Recently we also observed sandmining on large scale at Markal (Image 5), which is likely to affect the habitat of *G. poonaensis*.

Another major threat to the species could be the presence of introduced exotic fish. Introduced exotic fishes have been documented as major threats to fishes in the peninsular India (Kharat et al. 2003; Daniels 2006; Raghavan et al. 2008; Knight 2010). Kharat et al. (2003) have argued that introduced fish like *Oreochromis mossambica*, *Poecilia reticulata*, *Gambusia affinis*, *Heteropneustes fossilis*, etc. in Mula-Mutha River, are threatening the existence of many native fishes and might have caused even local extinction of some species including *G. poonaensis*.



**Image 5. Sandmining in Indrayani River at (a) Markal and (b) downstream of Markal is a potential threat to *G. poonaensis* habitat.**

Even in Indrayani River at Markal we recorded alien species such as *Oreochromis mossambica* and *Clarias gariepinus*. Although direct evidence is lacking we suspect that at least *Clarias gariepinus* is a potential threat to the current population of *G. poonaensis* as it is a voracious predator (Krishnakumar et al. 2011). Restricted distribution of the species, decline in the

extent of occurrence, ongoing threats to the habitats and possible threats to the species justifies the IUCN Red list threat category of this species as Endangered under the criteria B2ab(i,ii,iii,iv) as assessed by Dahanukar (2010).

The potential breeding grounds of the fish at Markal and the upstreams of Indrayani River, especially hill streams in the adjoining hilly areas need protection. Halting of siltation by re-plantations and avoidance of pollution could be helpful in saving the breeding grounds. Management of the introduced fishes, especially *Clarias gariepinus*, by controlled eradication of escaped stock and increasing public awareness for avoiding further introductions (Sato et al. 2010), could also be helpful.

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