

Table S1. Primers used to amplify and their sequences.

Locus	Primer	5'-3' primer sequence
COI	FishF1	TCAACCAACCACAAAGACATTGGCAC
	FishR1	TAGACTTCTGGGTGGCCAAAG AATCA
Cyt b	L14724	GACTTGAAAAACCACCGTTG
	H15915	CTCCGATCTCCG GATTACAAGAC
RAG2	MHRAG-2-F1	TGYTATCTCCACCTCTGCGYTACC
	MHRAG-2-R1	TCATCCTCCTCATCKTCTCWTGTGA

Table S2. List of different *Glyptothorax* species from outside the Middle East used in this study.

Species	COI	Cyt b	RAG2	Locality	Source
<i>G. aff. zanaensis</i> 1	HQ593582	HQ322531	HQ593556	China: Yunnan, Longling	[8]
<i>G. aff. zanaensis</i> 2	HQ593573	HQ322509	HQ593547	China: Yunnan, Lushui	[8]
<i>G. aff. zanaensis</i> 3	HQ593569	HQ322517	HQ593543	China: Yunnan, Baoshan	[8]
<i>G. burmanicus</i> 1	HQ593567	HQ593591	HQ593541	China: Yunnan, Longling	[8]
<i>G. burmanicus</i> 2	HQ593577	HQ322551	HQ593551	China: Yunnan, Gengma	[8]
<i>G. cf. lampris</i>	HQ593587	HQ593600	HQ593561	China: Yunnan, Lancang	[8]
<i>G. deqinensis</i>	HQ593564	HQ593590	HQ593538	China: Yunnan, Deqin	[8]
<i>G. hainanensis</i>	HQ593584	HQ593597	HQ593558	China: Hainan, Baisha	[8]
<i>G. honghensis</i>	HQ593583	HQ593596	HQ593557	China: Yunnan, Xiping	[8]
<i>G. interspinalum</i>	HQ593563	HQ593589	HQ593537	China: Yunnan, Lvchun	[8]
<i>G. laosensis</i>	HQ593565	HQ322523	HQ593539	China: Yunnan, Mengla	[8]
<i>G. longicauda</i>	HQ593578	HQ593593	HQ593552	China: Yunnan, Tengchong	[8]
<i>G. longjiangensis</i>	HQ593566	HQ322520	HQ593540	China: Yunnan, Longling	[8]
<i>G. macromaculatus</i>	HQ593568	HQ593592	HQ593542	China: Yunnan, Baoshan	[8]
<i>G. minimaculatus</i>	HQ593579	HQ322547	HQ593553	China: Yunnan, Tengchong	[8]
<i>G. ngapang</i>	HQ593571	HQ322514	HQ593545	China: Yunnan, Gengma	[8]
<i>G. obliquimaculatus</i>	HQ593576	HQ322553	HQ593550	China: Yunnan, Gengma	[8]
<i>G. pallozonum</i>	HQ593586	HQ593599	HQ593560	China: Guangdong, Haifeng	[8]
<i>G. quadriocellatus</i>	HQ593562	HQ593588	HQ593536	China: Yunnan, Lvchun	[8]
<i>G. siamensis</i>	EU490860	EU490909	DQ492335	Thailand	[10]
<i>G. sinensis</i> 1	HQ593585	HQ593598	HQ593559	China: Guangxi, Longlin	[8]
<i>G. sinensis</i> 2	HQ593580	HQ593594	HQ593554	China: Sichuan, Panzhihua	[8]
<i>Glyptothorax</i> sp. Cangyuan	HQ593570	HQ322516	HQ593544	China: Yunnan, Cangyuan	[8]
<i>Glyptothorax</i> sp. Nujiang	HQ593575	HQ322558	HQ593549	China: Yunnan, Fugong	[8]
<i>G. trilineatus</i> 1	HQ593581	HQ593595	HQ593555	China: Yunnan, Tengchong	[8]
<i>G. trilineatus</i> 2	HQ593572	HQ322512	HQ593546	China: Yunnan, Gengma	[8]
<i>G. zanaensis</i>	HQ593574	HQ322507	HQ593548	China: Yunnan, Gongshan	[8]
<i>Bagarius yarrelli</i> †	KM610423	AF416897	DQ192431	China	[11,12]

† Outgroup.

Table S3. GenBank accession numbers (seq. ID / GenBank number) for the new *Glyptothorax* material examined from the Middle East (details of the specimens are given in Table 1)

Species	COI	Cyt b	RAG2
<i>G. shapuri</i>		GY292 / OP589359	
<i>G. shapuri</i>	G396 / OP585111	P396 / OP589360	G396R / OP589382
<i>G. shapuri</i>	G970F / OP585112		
<i>G. shapuri</i>	G971F / OP585113		
<i>G. shapuri</i>	G333 / OP585114	GY333 / OP589361	
<i>G. shapuri</i>	G401 / OP585115	G401 / OP589362	G401R / OP589383
<i>G. silviae</i>	G395 / OP585116		
<i>G. silviae</i>	G410 / OP585117		
<i>G. silviae</i>		GY330 / OP589363	
<i>G. silviae</i>	G589 / OP585118	GY295 / OP589364	G589R / OP589384
<i>G. silviae</i>	G411 / OP585119	G411 / OP589365	G411R / OP589385
<i>G. hosseinpanahii</i>	G597 / OP585120	GY294 / OP589366	G597R / OP589386
<i>G. hosseinpanahii</i>	G598 / OP585121	GY332 / OP589367	G598R / OP589387
<i>G. alidaei</i>	Ex58F4 / OP585122		
<i>G. alidaei</i>	G606 / OP585123		
<i>G. alidaei</i>	Ex58F3 / OP585124		
<i>G. alidaei</i>	G414 / OP585125	G414 / OP589368	G414R / OP589388
<i>G. alidaei</i>		GY326 / OP589369	
<i>G. alidaei</i>		GY329 / OP589370	
<i>G. alidaei</i>	G728F / OP585126	G728b / OP589371	G835R / OP589389
<i>G. alidaei</i>	G726F / OP585127		
<i>G. alidaei</i>	G729F / OP585128		
<i>G. alidaei</i>	G727F / OP585129		
<i>G. cf. galaxias</i>	G323 / OP585130	GY323 / OP589372	G323R / OP589390
<i>G. cf. galaxias</i>	G558 / OP585131	G558b / OP589373	G558R / OP589391
<i>G. cf. galaxias</i>		G557b / OP589374	G557R / OP589392
<i>G. cf. galaxias</i>	G296 / OP585132	GY296 / OP589375	G296R / OP589393
<i>G. galaxias</i>		GY327 / OP589376	
<i>G. galaxias</i>		GY325 / OP589377	G331R / OP589394
<i>G. armeniacus</i>	G834 / OP585133		
<i>G. armeniacus</i>	G369 / OP585134		
<i>G. daemon</i> 1	G931F / OP585135		
<i>G. daemon</i> 1	G559 / OP585136		
<i>G. cous</i>	G324 / OP585137	GY324 / OP589378	G324R / OP589395
<i>G. cous</i>	G368 / OP585138	G368 / OP589379	G368R / OP589396
<i>G. kurdistanicus</i>	G510 / OP585139	G510b / OP589380	G510R / OP589397
<i>G. pallens</i>		G511b / OP589381	G511R / OP589398

Table S4. Results of the Xia's nucleotide substitution saturation test for COI, *Cyt b* and RAG2 of the studied *Glyptothorax* specimens

Locus	C.P.	<i>I</i> _{ss}	<i>I</i> _{ss.cS}	T	DF	P.S	<i>I</i> _{ss.cA}	T	DF	P.A
COI	1st + 2nd	0.054	0.695	17.511	39	0.0001	0.367	8.551	39	0.0001
	3rd	0.085	0.687	33.228	216	0.0001	0.368	15.612	216	0.0001
<i>Cyt b</i>	1st + 2nd	0.079	0.742	17.707	43	0.0001	0.483	10.793	43	0.0001
	3rd	0.085	0.669	40.035	328	0.0001	0.415	22.646	328	0.0001
RAG2	1st + 2nd	0.003	0.729	261.87	593	0.0001	0.512	183.53	593	0.0001
	3rd	0.012	0.666	95.840	296	0.0001	0.455	64.907	296	0.0001

C.P. = codon position; N.OTU = number of operational taxonomic units; *I*_{ss} = index of substitution saturation; *I*_{ss.cS} = critical value for symmetrical tree topology; T = T-value; DF = degrees of freedom; P.S and P.A. = probability that *I*_{ss} is significantly different from the critical value (*I*_{ss.cS} or *I*_{ss.cA}, respectively); *I*_{ss.cA} = critical value for extremely asymmetrical tree topology.