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Discovery of fossil Nemacheilids (Cypriniformes, Teleostei, Pisces) from the Tibetan Plateau, China

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Here described are the nemacheilid fossils from the Pliocene Lower Member of Qiangtang Formation in the Kunlun Pass Basin, northeastern Tibetan Plateau, at a locality 4769 m above the sea level (a. s. l.). The materials consist of numerous disarticulated and incomplete bones. The fish remains are assigned to the Nemacheilidae based on the fused compound centrum of the 2nd and 3rd vertebrae with developed bifurcate lateral processes of the 2nd vertebra. The fossils also include the maxilla, the dentary, the anguloarticular, the quadrate, the hyomandibular, the opercle, the basihyal, the urohyal, the anterior ceratohyal, the posterior ceratohval, the interhyal and the supracleithrum. These bones are very similar to their counterparts in some species of a Recent nemacheilid genus, Triplophysa (Nemacheilidae, Cypriniformes), which is widely distributed on the Tibetan Plateau. The nemacheilid fossils are much more abundant than the remains of schizothoracines embedded in the same horizon at the same locality. This would imply that the number of individuals of Triplophysa was much higher than that of schizothoracines when they were alive in the area. In Recent ichthyofauna of the Tibetan Plateau, Triplophysa prevails over schizothoracines in the number of individuals in the high elevations and small water bodies. Based on the fossil dominance of Triplophysa over schizothoracines and their taphonomical conditions, it appears that the water system at the Kunlun Pass area during the Pliocene might not be extensive lakes or large rivers with broad valleys. There might have been a few mountainous, relatively torrential rivers with many small, shallow streams connecting the water systems from the north and south of the East Kunlun Mountain. The environment of the Kunlun Pass Basin area during the Pliocene must be very harsh, and the altitude of the area might already have been higher than we previously suggested. The uplift of the area must be less than 1000 m since the Pliocene.

Nemacheilidae, Triplophysa, Pliocene, Kunlun Pass Basin, northeastern Tibetan Plateau

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We reported the Pliocene cyprinid fossils from the Lower Member of Qiangtang Formation in the Kunlun Pass Basin [1], northeastern Tibetan Plateau, and assigned them to *Gymnocypris*. Deemed as highly specialized schizothoracines [2], the living representatives of *Gymnocypris* still inhabiting cold waters at the high elevation in the upper reaches of the Yellow River and the Golmud River near the Kunlun Pass Basin. In this paper, we describe remains of another fish embedded in the same horizon at the same locality KL0607 where the fossil *Gymnocypris* was found, and this new form belongs to a different family of the Cypriniformes, i.e., the Nemacheilidae. In fact, the disarticulated bones are quite similar to their counterparts in *Triplophysa*, a living genus of the Nemacheilidae mainly distributed on the Tibetan Plateau, especially to the *Triplophysa* species from the upper reaches of the Yellow River and the Qaidam River near the Kunlun Pass Basin.

The systematic position of the Nemacheilidae is still in dispute. Traditionally, it was treated as a subfamily Nema-

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cheilinae of the family Cobitidae, along with the Cobitinae and Botinae [3–5]. Sawada [6] transferred the Nemacheilinae from the Cobitidae to the Homalopteridae (= Balitoridae) based on a few shared osteological characters between Nemacheilinae and Homalopterinae. However, further morphological study by Nalbant and Bianco [7] revealed that these similarities between the two groups were merely homoplasies, and raised the Nemacheilinae to the family level within the superfamily Cobitoidea. Current molecular studies [8, 9] came to similar conclusions. Although different taxonomic positions and ranks have been given to the Nemacheilidae, the group is always treated as monophyletic. In this paper we accept the current results of both morphological and molecular works.

The Nemacheilidae is the most diverse group in the Cobitoidea, and its generic classification is still under debate. Sawada [6] assigned most of nemacheilids from Eurasia to one enormous genus *Nemacheilus (sensu lato)*, but Zhu [10] and Prokofiev [11, 12] divided this group into dozens of separate genera. In this paper, we follow Zhu [10] that most of the Recent nemacheilids living in the Tibetan Plateau were assigned to the genus *Triplophysa* and those living in northern Eurasia were assigned to *Barbatula*.

1 Material and methods

The nemacheilid fossils were associated with the cyprinid fossils from the same locality KL0607 in the Kunlun Pass Basin, and all remains consist of disarticulated, incomplete bones. They do not seem buried *in situ* but sorted and transported by water, then re-deposited, for only relatively thick bones or thick parts of bones are preserved. Usually the bones of nemacheilids are brown and lighter in color than the black cyprinid bones, and all fossils stand out against the lighter-colored matrix. The fossils were easily picked out or screen-washed from the comparatively soft matrix and then cleaned mechanically using a thin needle under binocular WILD- M7A stereomicroscope. The specimens of nemacheilids are numbered as IVPP V 18019–18031.

Materials used for comparison include disarticulated bones prepared from ten species of Recent nemacheilids. They are: *Barbatula barbatula* IVPP OP 369 collected from Hebei; *Triplophysa siluroides* IVPP OP 367 from the upper reaches of the Yellow River, Sichuan; *T. stoliczkae* IVPP OP 368, *T. orientalis* IVPP OP 385, and *T. stewarti* IVPP OP 386 from Yalu Zangbu River, Xizang; *T. stenura* IVPP OP 387 from the upper reaches of the Yangtze River, Qinghai; *T. dalaica* IVPP OP 388 from Inner Mongolia; *T. leptosoma* IVPP OP 389 from the upper reaches of the Qaidam River, Qinghai; *T. scleroptera* IVPP OP 390 and *T. pseudoscleroptera* IVPP OP 391 from the upper reaches of the Yellow River, Gansu. Recent nemacheilid species were identified according to Zhu [10]. All specimens are stored in the collections of IVPP.

Photographs were taken with CANON 1Ds digital camera connected to OLYMPUS SZX12 stereomicroscope. Some bones of Recent nemacheilids were stained with alizarin red S. General osteological terminology mainly follows that of Sawada [6] and Prokofiev [12].

2 Systematic paleontology

Superorder Ostariophysi Sagemehl, 1885 Order Cypriniformes Bleeker, 1859/1860 Family Nemacheilidae Nalbant and Bianco, 1998 Genus *Triplophysa* Rendahl, 1933 *Triplophysa* sp. indet.

Referred specimens: IVPP V 18019.1–6, the compound centrum of the 2nd and 3rd vertebrae; IVPP V 18020.1–7, maxillae; IVPP V 18021.1–62, dentaries; IVPP V 18022.1–303, anguloarticulars; IVPP V 18023.1–73, quadrates; IVPP V 18024.1–140, hyomandibulars; IVPP V 18026.1–4, basihyals; IVPP V 18027.1, anteroventral parts of urohyals; IVPP V 18028.1–1288, anterior ceratohyals; IVPP V 18029.1–845, posterior ceratohyals; IVPP V 18030.1–8, interhyals; IVPP V 18031.1–13, supracleithrums.

Locality and horizon: KL0607 (35°38'09.0"N, 94°05'05.6"E), the Kunlun Pass Basin, Qinghai Province, China; Lower Member of the Qiangtang Formation, Pliocene.

3 Description

(1) The compound centrum of the 2nd and 3rd vertebrae. Six partially preserved compound centrums of the 2nd and 3rd vertebrae were picked out (IVPP V 18019.1-6). The centrum is amphicoelous, constricted in the middle and with the anterior portion broader than the posterior (Figure 1(a)-(d)). In the dorsal surface, there is an X-shaped ridge in the center, with a very prominent rear bifurcation, extending backwards to form the posterodorsal neural postzygapophyses of the centrum. A large fossa is situated on each lateral side of the ridge, probably for the articulation of the 3rd neural arch. The ventral surface of the centrum is depressed in the center, with a few scattered tiny pores and pits. Two processes protrude forward from the anteroventral margin of the centrum. The lateral processes of the 2nd vertebra are broken, only with the proximal portions preserved, which are broad and extending posteriorly. The horizontal and descending branches of the lateral processes are combined by poriferous bone. The two branches tend to form the bony capsule of the swim bladder as in Recent nemacheilid Triplophysa pseudoscleroptera (Figure 1(e), (f)). In Recent nemacheilids, the anterior parts of the bony capsules are formed by the horizontal



Figure 1 Compound centrum of the 2nd and 3rd vertebrae of fossil nemacheilid IVPP V 18019.1 ((a), (b)), IVPP V 18019.2 ((c), (d)) and the same vertebrae with bony capsule of swim bladder in Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((e), (f)). Bone of Recent nemacheilid was stained with alizarin red S. (a), (e) dorsal view; (b), (f) ventral view; (c) anterior view; (d) posterior view. The anterior facing up in (a), (b), (e) and (f). The dorsal facing up in (c) and (d). Abbreviations: ac, process from the anteroventral margin of the centrum; afn, articular fossa for the 3rd neural arch; bc, bony capsule; c, centrum; dl, descending branch of the lateral process; hl, horizontal branch of the lateral process; pnp, posterodorsal neural postzygapophyses. Scale bars: 0.5 mm for (a)–(d), 1 mm for (e) and (f).

and descending branches of the lateral processes of the 2nd vertebra [5, 6, 13].

(2) Maxilla. One left anterior, four left posterior and two right posterior parts of maxillae were picked out (IVPP V 18020.1-7). Specimen IVPP V 18020.1 (Figure 2(a), (b)) is the anterior part of a left maxilla and specimen IVPP V 18020.2 (Figure 2(d), (e)) is the posterior part of a right maxilla. The two specimens show that the maxilla is comparatively broad and, on the whole, plate-like, with two narrow sections, one behind the head and the other in front of the knob-like posterior end of the bone (Figure 2(c)). The process at the anterodorsal terminal of the bone, possibly for the attachment of the prepalatine and preethmoid II in this kind of fish, is well developed. The ventro-rostral process is situated at the anteroventral end of the bone and protrudes ventrally. The distal tip of the process is broken in specimen IVPP V 18020.1. A small lateral process is situated at the base of the ventro-rostral process, as in many Recent nemacheilids [12]. The dorsomedial margin of the maxilla is thin, whereas the ventrolateral margin of the bone is thickened. There is a prominent process at the ventral margin, posterolateral to the ventro-rostral process. Such process also exists in the maxilla of Recent nemacheilid Triplophysa pseudoscleroptera (Figure 2(f), (g)) but absent in fossil cyprinid maxilla embedded in the same horizon at the same locality. The ventral margin posterior to the process is sigmoid, with the rear end of the maxilla bent down and widened into a round knob. The dorsal margin of specimen IVPP V 18020.2 protrudes into a broad process.

Twenty-three left and 39 right nearly (3) Dentary. complete or partially preserved dentaries were picked out (IVPP V 18021.1-62). Specimen IVPP V 18021.1 is a nearly intact right dentary consisting of two parts: the slender anterior branch (ramus dentalis [12]) and the deep and widened coronoid process in the rear (Figure 3(a), (b)). The anterior branch bends slightly inward, with the thick front terminal bearing a deep joint facet to meet its counterpart. There is a tiny process situated posteriorly, proximate to the joint facet in the medial surface of the dentary. The dorsal surface of the anterior branch is narrow, with its lateral margin ridge-like. The coronoid process is nearly vertical with rounded dorsal border, and its front margin is concave. The lateral surface of the dentary is smooth, with no openings of sensory canal. The groove accomodating the insertion of the Meckel's cartilage and anguloarticular in the medial surface of the dentary originates in the rear of the anterior branch, and becomes broader posteriorly. The ventral margin of the dentary is slightly concave in the front. This fossil is similar to the dentary of Recent nemacheilid Triplophysa pseudoscleroptera (Figure 3(c), (d)) and can be easily distinguished from the fossil dentary of cyprinid embedded in the same horizon at the same locality and dentaries in cyprinids as a whole by the absence of openings of sensory canal in the lateral surface.

(4) Anguloarticular. One hundred and fifty-nine left and 144 right nearly complete or partially preserved anguloarticulars were picked out (IVPP V 18022.1–303). Specimen IVPP V 18022.1 is a well preserved left anguloarticular



Figure 2 Maxillae of fossil nemacheilid IVPP V 18020.1 ((a), (b)) and IVPP V 18020.2 ((d), (e)), restoration of the shape of the fossil maxilla (c) based on specimen IVPP V 18020.1 and IVPP V 18020.2, and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((f), (g)). Bones of Recent nemacheilid were stained with alizarin red S. (a), (b) and (f) left one; (d), (e) and (g) right one; (a), (d) and (f) lateral view; (b), (e) and (g) medial view. The anterior facing left in (a), (c) and (e)–(g). The anterior facing right in (b) and (d). Abbreviations: dpr, dorsal process; lvr, lateral process at the base of the ventro-rostral process; pk, posterior knob; pp, process for the attachment of the prepalatine and preethmoid II; pv, process at the ventral margin; vr, ventro-rostral process. Scale bars: 0.5 mm for (a)–(e); 1 mm for (f) and (g).

(Figure 4(a), (b)). It is a deep and short bone, with its anterior part tapering into a sharp tip for the insertion into the groove in the medial surface of the dentary. The posterior part of the anguloarticular is thickened, with an articular socket at the rear terminal for receiving the articular head of the quadrate. The joint facet for the retroarticular is situated on the ventral surface of this part. The lateral surface of the bone bears several pits scattering near the articular socket and several anteroposteriorly stretching grooves lying in front. No sensory canal opening can be found in the lateral surface of the anguloarticular. The medial surface of the bone is uneven, with a small projection, which must have been attached by the posterior end of the Meckel's cartilage. The dorsal margin of the anguloarticular is convex with an angular posterodorsal corner. The ventral margin of the bone is on the whole convex, with its anterior and posterior parts slightly concave. This fossil is similar to the anguloarticular of Recent nemacheilid *Triplophysa pseudoscleroptera* (Figure 4(c), (d)) and can be distinguished from the fossil anguloarticular of cyprinid embedded in the same horizon at the same locality by the absence of opening of sensory canal in the lateral surface.

(5) Quadrate. Thirty-six left and 37 right partially preserved quadrates were picked out (IVPP V 18023.1–73). Specimen IVPP V 18023.1 is the anterior part of a left quadrate (Figure 5(a), (b)) and specimen IVPP V 18023.2 is the ventral part of a right quadrate (Figure 5(c), (d)). The two specimens show that the quadrate has an anterodorsal plate and a posteroventral shaft. The anterodorsal plate is subtriangular, with several pits on its medial surface around



Figure 3 Right dentary of fossil nemacheilid IVPP V 18021.1 ((a), (b)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((c), (d)). Bone of Recent nemacheilid was stained with alizarin red S. (a) and (c) lateral view, the anterior facing right; (b), (d) medial view, the anterior facing left. Abbreviations: cp, coronoid process; ds, dorsal surface of anterior branch; gca, groove accomodating Meckel's cartilage and anguloarticular; j, joint facet for the counterpart; Im, lateral margin of dorsal surface of anterior branch; mp, medial process proximate to the joint facet. Scale bars: 1 mm for (a) and (b), 0.5 mm for (c) and (d).



Figure 4 Left anguloarticular of fossil nemacheilid IVPP V 18022.1 ((a), (b)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((c), (d)). Bone of Recent nemacheilid was stained with alizarin red S. (a), (c) lateral view, the anterior facing left; (b), (d) medial view, the anterior facing right. Abbreviations: jr, joint facet for the retroarticular; pc, projection attached by the posterior end of the Meckel's cartilage; sq, socket for articular head of quadrate. Scale bars: 1 mm for (a) and (b), 0.5 mm for (c) and (d).



Figure 5 Quadrate of fossil nemacheilid IVPP V 18023.1 ((a), (b)), IVPP V 18023.2 ((c), (d)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((e), (f)). Bones of Recent nemacheilid were stained with alizarin red S. (a), (b) and (e) left one; (c), (d) and (f) right one; (a), (c) and (e) lateral view; (b), (d) and (f) medial view. The anterior facing left in (a), (d)–(f). The anterior facing right in (b) and (c). Abbreviations: a, articular facet for anguloarticular; ad, anteroventral angle of dorsal plate; dpl, dorsal plate; v, posteroventral shaft. Scale bars: 0.5 mm for (a)–(d); 1 mm for (e) and (f).

the anteroventral corner. The anteroventral angle of the plate protrudes forward beyond the articular head for the anguloarticular, forming a deep notch between the plate and the articular head, as in Recent nemacheilid *Triplophysa pseudoscleroptera* (Figure 5(e) and (f)). In cyprinids, the dorsal plate of the quadrate does not have such prolonged, pointed anteroventral angle. The anterior articular facet for anguloarticular is broad and slightly depressed in the center, as in Recent nemacheilids, whereas the fossil quadrate of cyprinid embedded in the same horizon at the same locality have comparatively flat anterior articular facet for anguloarticular. The shaft-like posteroventral part of the quadrate tapers towards the rear. The lateral surface of the bone is smooth.

(6) Hyomandibular. Sixty-seven left and 73 right nearly complete or partially preserved hyomandibulars were

picked out (IVPP V 18024.1-140), and the specimen IVPP V 18024.1 is a nearly completely preserved left one (Figure 6(a), (b)). The bone is subtriangular, broad in its dorsal part and narrowing ventrally. The dorsal margin of the bone bears two separate protuberance-like articular areas for the neurocranium. The anterior one is round and bulged, whereas the posterior one is elongated and comparatively flat. As in Recent nemacheilid Triplophysa pseudoscleroptera (Figure 6(c), (d)), neither of the two areas reaches the front or rear terminal of the dorsal margin of the hyomandibular, and the rear terminal of the dorsal margin tapers into a sharp tip. The two articular areas for the neurocranium in the fossil are situated more apart from each other than those in Recent nemacheilid Triplophysa pseudoscleroptera. The articular condyle for the opercle protrudes from the posterior margin in the medial surface of the bone. Many



Figure 6 Left hyomandibular of fossil nemacheilid IVPP V 18024.1 ((a), (b)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((c), (d)). Bone of Recent nemacheilid was stained with alizarin red S. (a), (c) lateral view, the anterior facing left; (b), (d) medial view, the anterior facing right. Abbreviations: an, articular facet for neurocranium; ao, articular condyle for opercle; dna, dorsal opening for hyomandibular ramus of facial nerve and efferent hyoidean artery; vna, ventral opening for hyomandibular ramus of facial nerve and efferent hyoidean artery. Scale bars: 0.5 mm for (a) and (b), 1 mm for (c) and (d).

pits and short grooves are scattered on the medial surface of the bone and the dorsal opening of the passage for the hyomandibular ramus of the facial nerve (VII) and the efferent hyoidean artery is in front of the articular condyle. The ventral opening of the passage for the hyomandibular ramus of the facial nerve (VII) and the efferent hyoidean artery is situated near the ventral terminal on the lateral surface of the bone. There is a depressed area with several pits close to the dorsal margin on the lateral surface of the bone. The rear margin of the bone is concave near the articular condyle. The front margin in this specimen is broken. (7) Opercle. Two hundred and fifty-five left and 264 right opercles were picked out (IVPP V 18025.1–519), and only their anterodorsal parts are preserved like the specimen IVPP V 18025.1 (Figure 7(a), (b)). The opercular arm protrudes anterodorsally with a slightly thickened end, forming an obtuse angle with the dorsal margin of the bone. The articular fossa for the condyle of the hyomandibular is situated at the base of the opercular arm on the medial surface. The fossa is closer to the dorsal margin of the opercle than in that of the cyprinid embedded in the same horizon at the same locality. A horizontal ridge extends backward from



Figure 7 Right opercle of fossil nemacheilid IVPP V 18025.1 ((a), (b)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((c), (d)). Bone of Recent nemacheilid was stained with alizarin red S. (a), (c) lateral view, the anterior facing right; (b), (d) medial view, the anterior facing left. Abbreviations: ah, articular fossa for hyomandibular; am, anterior margin; dm, dorsal margin; hr, horizontal ridge; oa, opercular arm. Scale bars: 0.5 mm for (a) and (b), 1 mm for (c) and (d).

the articular fossa along the dorsal margin on the medial surface of the bone, as in Recent nemacheilid *Triplophysa pseudoscleroptera* (Figure 7(c), (d)). Pits arranged in rows radiating from the articular fossa are seen on both medial and lateral surfaces.

(8) Basihyal. Specimen IVPP V 18026.1 is a nearly complete basihyal (Figure 8(a), (b)). The bone is flat, consisting of a plate-like anterior part and a rod-like posterior part. Its anterior part bends slightly dorsally. There is a tiny lateral process on each side of the posterior part of the bone near the rear terminal, with a fossa situated between the two processes on the dorsal surface. The dorsal surface of the bone is flat with a shallow depression in the middle of the anterior part, whereas the ventral surface of the bone is smooth. This fossil is similar to the basihyal of Recent nemacheilid *Triplophysa siluroides* (Figure 8(c), (d)). Although the fossil basihyal of cyprinid has not been found in the Kunlun Pass Basin, its counterpart in Recent cyprinid is rod-like, not laterally expanded in the anterior part.

(9) Urohyal. Specimen IVPP V 18027.1 is the anteroventral part of an urohyal (Figure 9(a), (b)). This part is short, broad, and pentagon-shaped. It has a roughly straight anterior margin. The lateral margin is concave anteriorly, but protrudes outwards into a prominent angle posteriorly. Its posterior margin protrudes into two short spikes. There is a pair of depressed joint facets at the anteroventral rim of the bone for the connection with the ventral hypohyals by ligaments. The ventral surface of the bone is relatively smooth, with a few scattered pits. The posterodorsal part of the bone is lost. This fossil is similar to the anteroventral part of the urohyal of Recent nemacheilid *Triplophysa siluroides* (Figure 9(c), (d)). The fossil urohyal of cyprinid embedded in the same horizon at the same locality is in different shape [1].

(10) Anterior ceratohyal. Six hundred and fifty-three left and 635 right nearly complete or partially preserved anterior ceratohyals (following Conway et al.14], equivalent to the ceratohyal in Sawada [6] and Prokofiev [12])



Figure 8 Basihyal of fossil nemacheilid IVPP V 18026.1 ((a), (b)) and Recent nemacheilid *Triplophysa siluroides* IVPP OP 367 ((c), (d)). (a), (c) dorsal view; (b), (d) ventral view. The anterior facing left. Abbreviation: lp, lateral process. Scale bars: 0.5 mm for (a) and (b), 2 mm for (c) and (d).



Figure 9 Urohyal of fossil nemacheilid IVPP V 18027.1 ((a), (b)) and Recent nemacheilid *Triplophysa siluroides* IVPP OP 367 ((c), (d)). (a), (c) dorsal view; (b), (d) ventral view. The anterior facing left. Abbreviations: du, dorsal plate of urohyal; jh, joint facet for ventral hypohyal. Scale bars: 0.5 mm for (a) and (b), 2 mm for (c) and (d).

were picked out (IVPP V 18028.1–1288). Specimen IVPP V 18028.1 is a nearly complete right one (Figure 10(a), (b)). The bone is of rectangular shape, with its posterior end deeper and thinner than the anterior terminal, and the middle part is constricted. Its thick anterior end bears two joint facets for the two hypohyals to attach, one facing the front for the ventral hypohyal and the other facing dorsomedially for the dorsal hypohyal. The posterior edge of the bone is in connection with the anterior edge of the posterior ceratohyal.

Both the dorsal and ventral margins of the bone are concave, and the rear part of the ventral edge is rather thin. Both lateral and medial surfaces of the bone are covered with many pits. The lateral surface of the bone has a depression near the posteroventral corner for the attachment of the 2nd branchiostegal ray. There is a prominent deep fossa in the middle of the medial surface of the bone near its ventral margin for the attachment of the 1st branchiostegal ray. Such a prominent fossa is also present in the anterior cerato-



Figure 10 Right anterior ceratohyal of fossil nemacheilid IVPP V 18028.1 ((a), (b)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((c), (d)). Bone of Recent nemacheilid was stained with alizarin red S. (a), (c) lateral view, the anterior facing right; (b), (d) medial view, the anterior facing left. Abbreviations: dsb: depression for the 2nd branchiostegal ray; ffb, fossa for the 1st branchiostegal ray; jdh, joint facet for dorsal hypohyal; jvh, joint facet for ventral hypohyal. Scale bars: 1 mm for (a) and (b), 0.5 mm for (c) and (d).

hyal of Recent nemacheilid *Triplophysa pseudoscleroptera* (Figure 10(c), (d)), but absent in the anterior ceratohyal of cyprinid embedded in the same horizon at the same locality.

(11) Posterior ceratohyal. Three hundred and seventy-nine left and 466 right nearly complete or partially preserved posterior ceratohyals (following Conway et al. [14], equivalent to the epihyal in Sawada [6] and Prokofiev [12]) were picked out (IVPP V 18029.1-845). Specimen IVPP V 18029.1 is a nearly complete left one (Figure 11(a), (b)). The bone is of triangular shape, with a deep front margin in connection with the anterior ceratohyal and a posterodorsal process above the rear angle. The joint facet for the interhyal is situated at the posterodorsal edge of the bone, just in front of the posterodorsal process. Along the anterior two thirds of the dorsal margin of the bone is a narrow groove with foramina sitting on the bottom, possibly for the hyoidean artery to traverse. The ventral margin of the bone is blade-like. There are several short grooves stretching anteroposteriorly near the front edge on both lateral and medial surfaces of the bone. The lateral surface of the bone is depressed near the ventral margin, bordered by a horizontal ridge above it. This narrow depression must be for the attachment of the 3rd branchiostegal ray. The distinct depression on the lateral surface and the prominent posterodorsal process above the rear angle of the fossil posterior ceratohyal resemble that of Recent nemacheilid Triplophysa pseudoscleroptera (Figure 11(c), (d)). In contrast, the cyprinid posterior ceratohyal embedded in the same horizon at the same locality has a much shorter process above the rear angle and the depression attached by the third branchiostegal ray is limited at the anteroventral corner on the lateral surface of the bone.

(12) Interhyal. Three left and five right interhyals were picked out (IVPP V 18030.1–8). Specimen IVPP V 18030.1 is a right one (Figure 12(a), (b)). The bone is rod-like, with the joint facet with the hyomandibular and symplectic is flat and situated at the dorsal end of the bone. Its ventral end enlarges and bears a slightly concave facet for articulation with the posterior ceratohyal. The posterior tip of the ventral end of the bone is pointed. The lateral surface of the interhyal for adherence to the medial side of the preopercle is raised, whereas the medial surface of the interhyal is flat and depressed near the dorsal end. This fossil is similar to the interhyal of Recent nemacheilid *Triplophysa siluroides* (Figure 12(c), (d)) and in different shape to that of cyprinid embedded in the same horizon at the same locality [1].

(13) Supracleithrum. Six left and seven right supracleithrums were picked out (IVPP V 18031.1–13). Specimen IVPP V 18031.1 is a right one (Figure 13(a), (b)). The bone is slender, laminal, and somewhat sinuous, with its dorsal terminal blunt and bending slightly forward. There is a notch on each side of the dorsal terminal. The lateral surface of the dorsal part of the bone covered by the posttemporal is depressed, whereas the medial surface of this part is raised. The posteroventral edge of the dorsal part is thick-



Figure 11 Left posterior ceratohyal of fossil nemacheilid IVPP V 18029.1 ((a), (b)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((c), (d)). Bone of Recent nemacheilid was stained with alizarin red S. (a), (c) lateral view, the anterior facing left; (b), (d) medial view, the anterior facing right. Abbreviations: dtb, depression for the 3rd branchiostegal ray; gha, groove accommodating hyoidean artery; ji, joint facet for interhyal; rap, rear angle process. Scale bars: 1 mm for (a) and (b), 0.5 mm for (c) and (d).

ened, for the attachment of the ligament connecting to the distal end of the lateral process of the 1st centrum [15]. The ventral part of the supracleithrum curves, with its anterior margin convex and the posterior margin concave. The lateral surface of the ventral part of the bone is smooth, and the opening of the sensory canal is situated near the ventral tip in a narrow longitudinal groove beside the rear margin on the lateral surface. There is a broad longitudinal groove on the medial surface of the ventral part of the bone along its front margin, with foramina on the bottom. The ventral tip of the supracleithrum is pointed. This fossil is similar to the supracleithrum of Recent nemacheilid Triplophysa pseudoscleroptera (Figure 13(c), (d)), and the supracleithrum of cyprinid embedded in the same horizon at the same locality differs from that of nemacheilid in having a straight contour, more dorsally situated sensory opening, and no longitudinal groove on the medial surface. Furthermore, the structure of the supracleithrums of nemacheilids is more complicated than that of cobitids. For example, the supracleithrums of Cobitis and Misgurnus are nearly flat, with no longitudinal groove on the medial surface as in nemacheilids.

4 Discussion

4.1 Identification of fish remains

The Pliocene fish remains in the Lower Member of the Qiangtang Formation in the Kunlun Pass Basin described

above are assigned to the Nemacheilidae based on the fused compound centrum of the 2nd and 3rd vertebrae with developed bifurcate lateral processes of the 2nd vertebra. We tentatively assign the fossils to the nemacheilid genus Triplophysa because many of the bones bear similarities to their counterparts in Recent Triplophysa species living around the Kunlun Pass Basin (the Golmud River, the Qaidam River, upper reaches of the Yellow River and upper reaches of the Yangtze River). These include, the maxilla with a prominent process at the ventral margin, the dentary and the anguloarticular with no openings of sensory canal on the lateral surface, the quadrate with a protruding anteroventral angle, the hyomandibular with the rear terminal of the dorsal margin tapering into a sharp tip, the opercle with a horizontal ridge on the medial surface, the plate-like basihyal, the pentagon-shaped urohyal, the anterior ceratohyal with a prominent fossa in the medial surface, the posterior ceratohyal with prominent posterodorsal process above the rear angle, and the sinuous supracleithrum with a longitudinal groove on the medial surface (see above). The Nemacheilidae is the largest and taxonomically most complicated group (more than 40 genera and subgenera and more than 200 species and subspecies [10]) of the superfamily Cobitoidea, with its phylogenetic relationships insufficiently studied and its subdivision under debate [8, 9, 12]. Due to the large number of species and their wide distribution and the consequent difficulties in sample collection, very few comprehensive studies on morphology and anatomy-



Figure 12 Right interhyal of fossil nemacheilid IVPP V 18030.1 ((a), (b)) and Recent nemacheilid *Triplophysa siluroides* IVPP OP 367 ((c), (d)). (a), (c) medial view, the anterior facing left.; (b), (d) lateral view, the anterior facing right. Abbreviations: ahs, articular facet for hyomandibular and symplectic; apc, articular facet for posterior ceratohyal; pt, posterior tip of the ventral end. Scale bars: 0.5 mm for (a) and (b), 1 mm for (c) and (d).

apc

apc

of nemacheilids have been conducted, and still less on osteology [6, 11, 12]. No thorough study on osteology of the Chinese nemacheilids has ever been done so far, and the researchers on systematics of the Chinese nemacheilids rely mainly on external and soft tissue morphology [10], referring only to a few osteological characters [5, 13, 15]. With the most diverse nemacheilid genus *Triplophysa* (about 100 species and subspecies [16]), the only genus of the Nemacheilidae dwelling on Tibetan Plateau, the phylogenetic studies of most Chinese *Triplophysa* species are based on external and soft tissue morphology [17] and DNA sequence [16], only a few studies of the genus, and again only on a few species, even included osteological features [18, 19].

4.2 Fossil records of nemacheilids and their distribution

Fossil records of Nemacheilidae are rare, and only sparse late Cenozoic records were found in Europe, Central Asia,



Figure 13 Right supracleithrum of fossil nemacheilid IVPP V 18031.1 ((a), (b)) and Recent nemacheilid *Triplophysa pseudoscleroptera* IVPP OP 391 ((c), (d)). Bone of Recent nemacheilid was stained with alizarin red S. (a), (c) lateral view, the anterior facing right; (b), (d) medial view, the anterior facing left. Abbreviations: dp, dorsal part covered by the posttemporal; el, posteroventral edge of the dorsal part for the attachment of the ligament connecting to the distal end of the lateral process of the 1st centrum; lgm, longitudinal groove on the medial surface; osc, opening of sensory canal. Scale bars: 0.5 mm for (a)–(d).

and Mongolia [20]. The middle to upper Miocene fossil unearthed from Kirgizia was assigned to the genus *Triplophysa* on the basis of the male secondary sex characteristic that the 5th to 6th broadened anterior branched pectoral fin rays are covered by aggregations of breeding tubercles [20]. The Pliocene fossil nemacheilid unearthed from the Kunlun Pass Basin is the first fossil record of this group on the Tibetan Plateau. Prior to our findings there was no fossil record of nemacheilids from China. The presence of *Triplophysa* from the Miocene of Tibet mentioned by Prokofiev [20] and repeated by Conway et al. [21] was an incorrect quote from Wu and Wu [17]. We asked Wu and Wu about this question and they confirmed that they had never mentioned any finding of fossil *Triplophysa* (or nemacheilids) in any of their works on the fishes from the Tibetan Plateau and other parts of China (Wu and Wu, personal communication, 2010).

In the Recent ichthyofauna of Tibetan Plateau, the only representative genus Triplophysa of the Nemacheilidae usually lives together with schizothoracines (Cyprinidae) in cold waters there. Some Triplophysa species adapt to more severe habitats than schizothoracines, e.g., some small, shallow, and saline water bodies [17]. Although the Triplophysa is the only Recent representative genus of the Nemacheilidae living on the Tibetan Plateau, the distribution of Triplophysa is not as restricted on the Plateau and its surrounding areas as that of schizothoracines. Some Triplophysa species spread eastward to the central part of China at the altitudes of less than 1000 m a. s. l. [10]. In contrast to the fossil schizothoracines found abundantly from several localities on Tibetan Plateau [22], the fossil nemacheilid so far was known only from the Kunlun Pass Basin.

4.3 Development of water systems and paleoaltimetery

In Recent separated water systems around the Kunlun Pass Basin, namely the Qaidam inland drainage, the upper reaches of the Yellow River and upper reaches of the Yangtze River, several Triplophysa species, such as T. stoliczkae, T. microps and T. orientalis, inhabit all three of them [10, 17, 23]. This distribution pattern suggests that these Triplophysa species may have originated before the separation of these water systems. Furthermore, each water system around the Kunlun Pass Basin has its endemic Triplophysa species. For example, T. cakaensis is endemic to the Qaidam inland drainage, T. siluroides and T. pappenheimi to the upper reaches of the Yellow River, and T. stewarti and T. stenura to the upper reaches of the Yangtze River [10, 17, 23]. These endemic species may implicate the diversification of Triplophysa species after the separation of the three closely situated water systems.

It is interesting to note that the number of *Triplophysa* bones found from the Kunlun Pass Basin is much higher than the number of those of *Gymnocypris* or schizothoracines embedded in the same horizon at the same locality. For instance, collected from the locality KL0607 were 653 left and 635 right anterior ceratohyals, 379 left and 466 right posterior ceratohyals, fragments of 255 left and 264 right opercles, 159 left and 144 right anguloarticulars, 67 left and 73 right fragments of hyomandibulars, and 36 left and 37 right quadrates of *Triplophysa*, as compared to only

15 left and 15 right anterior ceratohyals, 12 left and 11 right posterior ceratohyals, fragments of seven left and 11 right opercles, 26 left and 29 right anguloarticulars, seven left and 19 right hyomandibulars, and eight left and six right quadrates of schizothoracines [1]. The disarticulated bones of both Triplophysa and schizothoracines were picked out from the same rock samples, which were gathered randomly, without bias in collection. The number of remained bones depends merely on the thickness and hardness of the bones or parts of bones, i.e., the thicker the bones the more likely they tend to be preserved. As we know, the size of schizothoracines is on the whole much larger than that of nemacheilids, and the bones of the former are much stronger and more robust than those of the latter. The prevalence of the number of bones of Triplophysa at this locality may most probably imply that the number of individuals of its population was much higher than that of schizothoracines when they were alive alongside in the area. This, in turn, indicates that the environment was, on the whole, more favorable for Triplophysa than for schizothoracines to survive. In other words, in addition to the river system, where schizothoracines used to dwell, there might have been many small, shallow streams around, in which Triplophysa alone was adapted to live. It is true that there are countless serrated dorsal fin spines and pharyngeal teeth of schizothoracines (more than 1000 pieces, at least) unearthed from the locality [1]. This is probably because of these bony parts of schizothoracines are much more robust and can endure subsequent transportations by water and weathering, and those of nemacheilids are thin and fragile and thus difficult to be preserved as fossils. We have never found a single fin ray or pharyngeal tooth of Triplophysa in our materials. This also means that the real size of the populations was much larger than the meager fossils may have represented.

In Recent ichthyofauna of the Tibetan Plateau, on the whole, schizothoracines are most diverse and abundant, followed by nemacheilids [17, 24]. However, some Triplophysa species have a greater adaptability to the colder environment with shallow waters than schizothoracines, and thus have a wider distribution to the higher altitude (e.g., T. microps, up to 5600 m a.s.l.) than the latter [24]. In small water systems of certain high altitude areas on the Tibetan Plateau, nemacheilids prevail over schizothoracines in the number of individuals (W. Cao and Q. Tang, pers. comm., 2011). Judged by the obvious prevalence of quantity of bones of Triplophysa over schizothoracines in the Kunlun Pass Basin and the taphonomical conditions of the bones as being "sorted and transported by water, then re-deposited" [1], we can also deduce that during the Pliocene the water system here may not be the kind of extensive lake or large river with broad valley, which are usually situated in comparatively lower areas of the Plateau. It is more reasonable, therefore, to suggest that along with mountainous, relatively torrential river system that connects the water systems from the north and south of East Kunlun Mountain, i.e., those of the Qaidam Basin and the Yellow River, there might also be a network of small streams, fitting for *Triplophysa* to survive.

In our previous paper on *Gymnocypris* from the Kunlun Pass Basin [1], we suggested that the area has uplifted approximately 1000 m since the Pliocene, based on the comparison of the present altitude of the fossil locality with the altitude range of the habitats of the living species of the genus. On account of the presence of more *Triplophysa* remains than those of schizothoracines found from the locality, the environment of the Kunlun Pass Basin during the Pliocene must be more severe, and the water system less developed than we deduced from *Gymnocypris* alone. Consequently, the altitude in the area of the Kunlun Pass Basin during the Pliocene might already have been considerably high, higher than we proposed before [1]. In other words, the area has most probably elevated less than 1000 m since the Pliocene.

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- Wang N, Chang M. Pliocene cyprinids (Cypriniformes, Teleostei) from Kunlun Pass Basin, northeastern Tibetan Plateau and their bearings on development of water system and uplift of the area. Sci China-Earth Sci, 2010, 53: 485–500
- 2 Cao W, Chen Y, Wu Y, et al. Origin and evolution of schizothoracine fishes in relation to the upheaval of the Qinghai-Xizang Plateau (in Chinese with English abstract). In: Comprehensive Scientific Expedition to the Qinghai-Xizang Plateau, Chinese Academy of Sciences, ed. Studies on the Period, Amplitude and Type of Uplift of the Qinghai-Xizang Plateau. Beijing: Science Press, 1981. 118–130
- 3 Berg L S. Fauna of Russia and Adjacent Countries. Volume 3. St. Petersburg: Imperial Academy of Sciences, 1912. 369–704
- 4 Ramaswami L S. Skeleton of cyprinoid fishes in relation to phylogenetic studies. V. The skull and the gasbladder capsule of the Cobitidae. Proc Natl Inst Sci India, 1953, 19: 323–347
- 5 Chen J, Zhu S. Phylogenetic relationships of the subfamilies in the loach family Cobitidae (in Chinese with English abstract). Acta Zootaxon Sin, 1984, 9: 201–208
- 6 Sawada Y. Phylogeny and zoogeography of the superfamily Cobitoidea (Cyprinoidei, Cypriniformes). Mem Fac Fish Hokkaido Univ, 1982, 28: 65–223

- 7 Nalbant T T, Bianco P G. The loaches of Iran and adjacent region with description of six new species (Cobitoidea). Ital J Zoolog, 1998, 65: 109–123
- 8 Tang Q, Liu H, Mayden R, et al. Comparison of evolutionary rates in the mitochondrial DNA cytochrome b gene and control region and their implications for phylogeny of the Cobitoidea (Teleostei: Cypriniformes). Mol Phylogenet Evol, 2006, 39: 347–357
- 9 Slechtova V, Bohlen J, Tan H H. Families of Cobitoidea (Teleostei; Cypriniformes) as revealed from nuclear genetic data and the position of the mysterious genera *Barbucca*, *Psilorhynchus*, *Serpenticobitis* and *Vaillantella*. Mol Phylogenet Evol, 2007, 44: 1358–1365
- 10 Zhu S. The Loaches of the Subfamily Nemacheilinae in China (in Chinese with English abstract). Nanjing: Jiangsu Science and Technology Publishing House, 1989. 1–150
- 11 Prokofiev A M. Problems of the classification and phylogeny of Nemacheiline loaches of the group lacking the preethmoid I (Cypriniformes: Balitoridae: Nemacheilinae). J Ichthyol, 2009, 49: 874– 898
- 12 Prokofiev A M. Morphological classification of loaches (Nemacheilinae). J Ichthyol, 2010, 50: 827–913
- 13 Wu X, Chen Y, Chen X, et al. A taxonomical system and phylogenetic relationship of the family of the suborder Cyprinoidei (Pisces). Sci Sin, 1981, 24: 563–572
- 14 Conway W K, Chen W J, Mayden L R. The "Celestial Pearl danio" is a miniature Danio (s.s) (Ostariophysi: Cyprinidae): Evidence from morphology and molecules. Zootaxa, 2008, 1686: 1–28
- 15 Zhu S. A comparative study on the air-bladder and its bony capsule nemacheiline fishes (Cobitidae) in China (in Chinese with English abstract). Acta Hydrobiol Sin, 1986, 10: 137–143
- 16 He D, Chen Y, Chen Y. Research on molecular phylogeny and biogeography of the *Triplophysa* species (in Chinese). Prog Nat Sci, 2006, 16: 1395–1404
- 17 Wu Y, Wu C. The fishes of the Qinghai-Xizang Plateau (in Chinese with English abstract). Chengdu: Sichuan Publishing House of Science and Technology, 1992. 1–599
- 18 Prokofiev A M. Redescription of *Triplophysa alticeps* (Herzenstein, 1888), the type species of the subgenus *Qinghaichthys* Zhu, 1981, with notes on its taxonomic position. J Ichthyol, 2006, 46: 570–581
- 19 Prokofiev A M. The morphology and relationships of the loach *Triplophysa coniptera* (Teleostei, Balitoridae, Nemacheilinae). Zool Zhurnal, 2007, 86: 1102–1112
- 20 Prokofiev A M. Redescription of a fossil loach *Triplophysa opinata* (Yakowlew, 1959) from the Miocene of Kirgizia (Balitoridae: Nemacheilinae). J Ichthyol, 2007, 47: 26–31
- 21 Conway K W, Hirt M V, Yang L, et al. Cypriniformes: systematics and paleontology. In: Nelson J S, Schultze H P, Wilson M V H, eds. Origin and Phylogenetic Interrelationships of Teleosts. Munchen: Verlag Dr Friedrich Pfeil, 2010. 295–316
- 22 Chang M, Miao D, Wang N. Ascent with modification: fossil fishes witnessed their own group's adaptation to the uplift of the Tibetan Plateau during the late Cenozoic. In: Long M, Gu H, Zhou Z, eds. Darwin's Heritage Today: Proceedings of the Darwin 200 Beijing International Conference. Beijing: Higher Education Press, 2010. 60–75
- 23 Wu Y, Yu D, Wu C, et al. A preliminary study on the resources of fishes and conservation in Hohxil (Kokoxili) region of Qinghai Province (in Chinese with English abstract). Chin J Zool, 1994, 29: 9–17
- 24 Zhang C, He D. Fishes of Xizang (in Chinese). Bull Biol, 1997, 32: 9–10