Ossifications associated with the gill filaments of members of the Balitoridae and Cobitidae are described for the first time. Although gill-filament ossifications are common in teleosts, similar ossifications were not observed in other members of the order Cypriniformes. Their presence is interpreted as a shared and derived character uniting the families Balitoridae and Cobitidae as a monophyletic group.

Key words: branchial arches; Cypriniformes; ossification; primary lamellae.

Members of the cypriniform families Balitoridae and Cobitidae are small fishes, commonly referred to as loaches, found throughout the fresh waters of Eurasia and parts of northern and eastern Africa (Nalbant, 1963; Kottelat, 1990; Kottelat & Freyhof, 2007). Anatomical studies investigating the gill-filament morphology of balitorid and cobitid fishes have focused entirely on the size, number and distribution of secondary lamellae and make no reference to the endoskeleton of the filament (Robotham, 1978; Singh et al., 1981). During recent investigations of the osteology of cypriniformes, a previously undocumented feature of the gill filaments of balitorids and cobitids was discovered: the presence of a single ossification at the base of the cartilage rod associated with the afferent artery of the gill filament. This paper serves to describe these ossifications, documenting, for the first time, the presence of gill-filament ossifications within the order Cypriniformes.

Specimens were cleared and double stained for bone and cartilage study following the procedure outlined in Taylor & Van Dyke (1985). Gill arches were dissected from cleared and stained specimens, following the dissection protocol of Weitzman (1974), under a Leica S8AP0 stereomicroscope (www.leica.com). The first holobranch was then carefully removed from the first gill arch (right-side only) and photographed in a fanned out position under a glass cover slip using a Leica DFC280 mounted on the aforementioned microscope. Usage of the family group name Cobitidae follows that

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of Nalbant (2002) and Šlechtová et al. (2006, 2007) and is equal to the subfamily group name Cobitinae of earlier authors (Nalbant, 1963; Sawada, 1982). A complete list of all cypriniform materials examined is provided in Appendix I. Collection abbreviations follow Leviton et al. (1985), except for CMK (collection of M. Kot-Kotelat), Cornol and SLU (Saint Louis University Ichthyology Collection).

The gill filaments of cypriniform fishes are long and taper towards the tip, fitting the flight-feather pattern of Johnson & Patterson (1993). As is typical for teleosts, each gill filament is supported by an unbranched cartilaginous rod, which runs the length of the curved inner margin of the filament, adjacent to the afferent artery. The base of the cartilage rod is v-shaped. In members of the Balitoridae and Cobitidae, an ossification is associated with the v-shaped base of the cartilage rod of the longer filaments situated on the lateral edges of the branchial arches [Fig. 1(a)–(d)]. In balitorids, this ossification is a short rod that surrounds the base and lower outer edge of the cartilage rod [Fig. 1(a), (b)]. Only one species of balitorid, Protomyzon griswoldi (Hora & Jayaram), was found to lack such ossifications and only one member of the family [Barbatula toni (Dybowski), a member of the subfamily Nemacheilinae] was found to exhibit a type of gill-filament ossification different from that described above. In B. toni the gill-filament ossification is a thin pericondral lamina, situated at the fork of the v at the base of the cartilage rod [Fig. 1(c)], much smaller than the gill-filament ossifications of other nemacheilids examined [Fig. 1(b)]. Members of the family Cobitidae examined exhibit gill-filament ossifications similar to those described for B. toni [Fig. 1(d)].

No other species of cypriniform examined exhibited ossifications in association with the gill-filament base similar to those present in members of the Balitoridae and Cobitidae [Fig.1(e), (f)]. Only Gyrinocheilus pennocki (Fowler) had gill-filament ossifications, in the form of small intermittent, endochondral ossifications, around the base and along the lower third of the cartilaginous rod of the largest gill filaments, situated along the lateral edges of the branchial arches [Fig. 1(f)]. These ossifications are clearly different from the ossifications associated with the gill filaments of balitorids and cobitids. In balitorid and cobitid loaches, the gill-filament ossification is restricted to the base and lower outer edge of the cartilaginous rod. In this respect, the gill-filament ossifications of balitorids and cobitids are strikingly similar to those of the percid (Branson & Ulrikson, 1967) [Fig. 1(g)] and gasterosteoid fishes (Johnson & Patterson, 1993) [Fig. 1(h)]. In G. pennocki, each gill filament exhibits numerous small ossifications throughout the base and lower third of the cartilaginous rod that runs along the curved inner margin of the filament. Similar ossifications to those observed in G. pennocki are widespread amongst lower teleosts [Bijtel, 1951; Laurent, 1984 (referred to as a gill rod)] and were observed in the characiform Hoplias malabaricus (Bloch), the momyrid Hyperopisus bebe (Lacépède) and the elopomorph Elops senegalensis Regan, but not in the non-teleost, Amia calva L.

Johnson (1986) and Johnson & Patterson (1993) utilized derived features of the gill filaments to support the placement of Acanthocybium Gill with the billfishes and Indostomus Prashad & Mukerji with the Syngnathoidea, respectively [but see Britz & Johnson (2002) for an alternative hypothesis on the placement of Indostomus]. Though the modifications of the gill filaments of balitorids and cobitids described above are not as striking as those documented for certain scombroids or syngnathoids, they are clearly unique among cypriniforms. It is hypothesized that the presence of an ossification at the base and lower outer edge of the gill filament is a derived feature,
Fig. 1. Gill filaments (anterior holobranch, right side, anterior view) of cleared and stained specimens: (a) *Gastromyzon cranbrooki*, UAIC 141288.01, 32.7 mm standard length ($L_S$), (b) *Mesoneoemacheilus triangularis*, UAIC 14180.57, 64.0 mm $L_S$, (c) *Barbatula toni*, UAIC 14183.19, 52.5 mm $L_S$, (d) *Acanthopsis choirollhynchos*, AMNH 58307, 128.6 mm $L_S$, (e) *Chromobotia macracanthus*, AMNH 77904, 69.7 mm $L_S$, (f) *Gyrinocheilus pennocki* UAIC 14167.17, 78.2 mm $L_S$, (g) *Etheostoma microperesa* SLU uncat., 28.0 mm $L_S$ and (h) *Gasterosteus aculeatus* SLU uncat., 58.0 mm $L_S$. 

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i.e. synapomorphy, uniting the families Balitoridae and Cobitidae as a monophyletic grouping, a hypothesis that should be tested with additional representatives of these families and other cypriniform taxa.

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References


APPENDIX I

The following cypriform material was examined (the number of cleared and stained specimens are listed in parentheses).

**Balitoridae: Balitorinae:** Beaufortia cf. leveretti – UAIC 14166.06 (1); Gastromyzon borneensis – BMNH 2001.1.21.58-71(2); G. cranbrooki – UAIC 14288.01 (1); G. punctatus – UAIC 14289.01 (1); Gastromyzon sp. – UAIC 14166.21 (1); Halmagloptera stephensoni – BMNH 2001.1.15.853-872 (3); H. zollingeri – BMNH 2001.1.15.878-903 (3); Protomyzon griswoldi – BMNH 2001.1.21.116-119 (1); Sewellia lineolata – UAIC 14169.43 (1). **Nemacheilinae:** Acanthocobitis cf. zonalternans – UAIC 14166.01. (1); Barbatula barbatula – AMNH 37608 (4); Lefua costata – UAIC 14183.15 (1); Microneumacheilus taeniatus – AMNH 48937 (2).

**Botiidae:** Botia striata – UAIC 14180.14 (2); Chromobotia macracanthus – AMNH 77904; Sinibotia superciliaris – UAIC 14180.98 (2); Synchosus cf. berdmorei – UAIC 14181.01 (2); Yasuhikotakia modesta – UAIC 14181.04 (2); Y. morleti – UAIC 14181.05 (2); Y. sidthimunki – UAIC 14166.48 (2).

**Catostomidae:** Catostomus catostomus – UAIC 14171.01 (2); C. commersoni – UAIC 14172.01 (2); Ictiobus sp. – UAIC 14173.01 (1); Minytrema melanops – UAIC 14187.01 (2).

**Cobitidae:** Acanptopsis choirorhynchus – AMNH 58307 (1); Cobitis pacifica – UAIC 14183.06 (1); C. taenia – UAIC 14180.17; C. tetrilineata – UAIC 14183.01 (1); Iksookimia koreensis – UAIC 14183.08 (1); I. longicorpa – UAIC 14183.10 (1); Koreocobitis rotundicautada – UAIC 14183.13 (1); Lepidocephalichthys guntea – UAIC 14180.56 (2); Niwaella multifasciata – UAIC 14183.15 (1).

**Cyprinidae:** Abramis brama – AMNH 37594 (2), UMMZ 184987 (2); Acheilognathus cyanostigma – UMMZ 187566 (1); Agosia chrysogaster – KU 8084 (3); Alburnus alburnus – UMMZ 174614 (1); Alburnoides bipunctatus – UMMZ 184991 (1); Amblypharyngodon mola – UMMZ 187844 (2); Aphocypris chinensis – UMMZ 167397 (1); Aspius aspius – UMMZ 1746907 (1); Barbus barbus – AMNH 54635 (3); ‘Barbus’ bynni – AMNH 215380 (3); ‘B.’ paludinosus – AMNH 217300 (3); Blicca bjoerkna – AMNH 37599 (2), UMMZ 174617 (1); Boraras brichtae – BMNH 2004.4.26.18-21 (3); B. maculatus – BMNH 1995.5.17.112 – 126 (6); B. merah – BMNH 2004.4.26.10 – 17 (4); B. micros – BMNH 2004.4.29.1–3 (2); B. urophthalmoides – BMNH 2004.4.26.2–9 (2); Campostoma anomalous – AMNH 40260 (1); Chelaethiops bibie – BMNH 2006.3.9.46–93 (4), UMMZ 166632 (1);
Chondrostoma nasus – UMMZ 185029 (2); Clinostomus elongatus – AMNH 45955 (5); Couesius plumbeus – AMNH 41266 (5); Culter alburnus – UMMZ 66525 (2); Cyclocheilichthys apogon – BMNH 2001.1.15.699–718 (2); Cyprinella analostana – UAIC 11003.01 (2); C. labrosa – KU 88319 (2); C. proserpina – UAIC 8354.01 (2); Cyprinodorus elongatus – AMNH 45955 (5); Couesius plumbeus – AMNH 41266 (5); Culter alburnus – UMMZ 66525 (2); Cyclocheilichthys apogon – BMNH 2001.1.15.699–718 (2); Cyprinella analostana – UAIC 11003.01 (2); C. labrosa – KU 88319 (2); C. proserpina – UAIC 8354.01 (2); Cyprinus carpio – AMNH 49088 (1); Danio albolineatus – UMMZ 70708 (2); D. choprai – UAIC 14166.09 (2); D. erythromicron – UAIC 14166.23 (2); D. margaritatus – BMNH 2001.10.9.15–16 (2); D. nigrofasciatus – UAIC 14166.12 (2); D. rerio – BMNH 2001.3.12.76–92 (3); BMNH 1983.7.11.15–29 (2); Devario devario – UAIC 14166.18 (1); UMMZ 187873 (1); Diodon episcopa – KU 7427 (7); Engraulicypris sardella – AMNH 31917 (5); Erimystax x-punctatus – KU 18012 (3); E. metallicus – BMNH 2000.6.10.8031 – 8258 (3); Exoglossum maxilligual – KU 18925 (11); Garra dembeensis – BMNH 1984.9.7.50–60 (2); Hampala macrolepidota – BMNH 2000.6.10.7891–7900 (1); Hemitrema flammee – KU 18884 (10); Hesperoleucus symmetricus – KU 18917 (15); Hypophthalmichthys molitrix – AMNH 10222 (1); Ischikauia steenackeri – UMMZ 187564 (1); Leptocypris niloticus – BMNH 2006.3.9.108–162 (4); Leucaspis delineatus – UMMZ 160942 (1); Luxilus chrysocephalus – KU 12654 (2); L. pilsbryi – KU 15281 (8); Macrhybopsis gelida – KU 8111 (1); Microphysogobio labeoides – AMNH 10588 (4); Microrasbora cubotai – BMNH 2004.6.25.6–10 (3); M. nana – BMNH 2004.6.25.1–5 (3); M. rubescens – BMNH 2004.6.25.11–13 (2); UAIC 14297.01 (1); Notemigonus crysoleucas – KU 1357 (1); Notropis altipinnis – UAIC 7960.03 (10); N. buccatus – KU 17764 (4); N. buccula – KU 14286 (6); N. dorais – 11166.02 (2); N. harperi – KU 21251 (5); N. nubilus – KU 17189 (8); N. ortenburgeri – KU 12462 (5); Opsarichthys bidens – UMMZ 64240 (1); Parachela hypophthalmus – BMNH 48924 (2); Paralabuca roriveri – UMMZ 181128 (1); Phenacobius mirabilis – KU 7918 (4); P. teretulus – KU 18929 (17); P. uranops – KU 19619 (2); Phoxinus erythrogaster – UAIC 14176. (7); P. neogaeus – KU 18882 (12); P. phoxinus – AMNH 36873 (4); Petroleuciscus borysenichus – UMMZ 185112 (2); Pteronotropis welaka – KU 18895 (9); Ptychocheilus grandis – KU 18920 (12); Romanogobio albipinnatus – UMMZ 185111 (1); Rasbora argyrotaenia – UMMZ 157150 (5); R. cephalotaenia – BMNH 2000.10.18. 34–40 (2); R. spiloreca – UAIC 14185 (4); R. sumatrana – BMNH 2001.1.15. 6780 – 6801 (2); R. tornieri – BMNH 2000.7.9.63–81 (2); Rasboroides vaterifloris – BMNH 2004.6.25.26–30 (3); Rhinichthys evermanni – KU 18910 (7); Rhodes sericeus – AMNH 39117 (2); UMMZ 18511 (2); Rutilus rutilus – AMNH 36897 (4); Saltophasia baccala – UMMZ 187849 (1); Semotilus corporalis – KU 18854 (9); Squalidus japonicus – UMMZ 142961 (1); Sundanadaceo albipinnatus – UMMZ 185111 (1); Telestes souffia – UMMZ 185042 (1); UMMZ 188890 (4); Trigonostigma heteromorpha – BMNH 2004.6. 25.26–30 (2); Yuriria alta – KU 21247 (8).

Gyrinocheilidae: Gyrinocheilus aymonieri – BMNH 2000.6.11.3518-3555 (1); G. pennocki – UAIC 14167.17 (1), UAIC 14180.51 (2).