Quantitative variation and morphotype of intermuscular bones in Sinocyclocheilus cavefish

Kunfeng Yang¹,², Xiaofu Pan¹, Xiaoi Wang¹, Qian Liu¹, Junxing Yang¹,*

¹ State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
² University of Chinese Academy of Sciences, Beijing 100049, China

* Correspondence: yangjx@mail.kiz.ac.cn

Abstract: The cyprinid genus Sinocyclocheilus is an emerging cavefish model endemic to the massive southwestern karst area adjacent to the Qinghai-Tibetan Plateau of China. The development of the intermuscular bones in this species still has not been investigated so far. To explore the quantitative variation and morphotype of intermuscular bones in this unique species, we accounted the intermuscular bones number of Sinocyclocheilus fishes using X-ray screening method. The results indicate that the total number of intermuscular bones is from 84 to 120, the number of epineural is 51 to 78, and the number of epipleural is 32 to 44. The different parts of fish body have different number of intermuscular bones. The intermuscular bones number of the region before the first basipterygium of dorsal fin is from 4 to 18, the number of the region between the first basipterygium of dorsal fin and the first basipterygium of anal fin is from 52 to 72, and the number of the region after the first basipterygium of anal fin is from 27 to 39. It has been found that the Sinocyclocheilus fishes have two categories (epineural and epipleural) and six forms of intermuscular bones which including non-forked, one-end-unequal-bi-fork, one-end-equal-bi-fork, one-end-multi-fork, two-end-bi-fork, and two-end-multi-fork. Three species living in the cave environment for a long time, they have a less number of intermuscular bones. This suggests that specific cave life of Sinocyclocheilus cavefish may result in decreased intermuscular bone number. Our research provides valuable data for better understanding of the intermuscular bones development of Sinocyclocheilus cavefish.

Keywords: Intermuscular bones; Quantitative variation; Sinocyclocheilus fishes

Introduction:

Sinocyclocheilus, the riches genus of Chinese Cyprinidae fishes in terms of species diversity, has more than 60 nominal species (Zhao & Zhang, 2009). Some of them are troglobites with typical adaptive characteristics, and some of them are troglophilous without special adaptations to cave life (Wang & Chen, 1999). Their distribution are limited to various karst areas in Yunnan and Guizhou Provinces, and the Guangxi Zhuang Autonomous Region (Zhang et al., 2003). In recent years, several Sinocyclocheilus fishes were successfully bred by Phylogenetics & Biogeography research group of Kunming Institute of Zoology, Chinese Academy of Sciences (Pan et al. 2009a; 2009b; Yang et al. 2007; Pan et al. 2013; 2014).

Due to dark environment, the cavefish evolves a serious of adaptive characteristics including constructive and regressive features such as bone development. The intermuscular bones (IB), occur only in teleoste amongst recent vertebrates, are segmental, serially homologous ossification in the myosepta (Patterson & Johnson, 1995). According to the site of attachment in fish body, IB can be divided into three categories, epineural, epicentral and epipleural. Epineurals is above the horizontal septum and attach to the neural arches(Owen, 1866), epipleurals is located below the epineurals but attach to the hemal arches or ribs(Owen, 1846) and epicentals on it and attach to the central vertebrae(Owen, 1866). Intermuscular bone has gradually been lost during fish evolution, such as in a few teleosts there are only have epineural and epipleural, and in the spiny perciformes it has almost completely disappeared (Patterson & Johnson, 1995).

Several studies have described the developmental process of IB during early stage of life. (Meng & Su, 1960; Deng, 1959; Bing, 1962; Li & Wang, 1987). Patterson and Johnson (1995) analyzed the morphology of IB systematically from 125 genera of teleosts. The IB numbers have also been accounted in many species. So far, there are two main methods for counting IB number. The first is cooking the fish and dissecting out the IB (Dong et al., 2006; Lv et al., 2007; Ke et al., 2008; Lv et al., 2012); the second method is using X-ray photograph method (Vallod & Arthaud, 2009; von Sengbusch, 1963; von Sengbusch & Meske, 1968; Meske, 1968; Kossmann, 1972). According to Dong’s research (2006), they found that the IB number, although very similar sometimes, was not always the same in both sides of the body. There are more bones in the front dorsal part while bone number was almost equal in rear dorsal part and rear abdominal part. In genetic breed, the IB research of different ploidies indicates that enhancing varieties of fish with less IB can be produced by artificial methods, such as distant hybridization (Li et al., 2013).

The function of IB has not yet been understood. So far, several hypothesis have been proposed such as supporting the muscles in the both sides of body (Deng, 1959), transmitting muscle strength between sarcomeres (Dong et al., 2006; Lv et al., 2007; Deng, 1959; Johnson & Patterson, 2001); and enhancing the strength of herbivorous fishes at some extent (Daons & Staab, 2010). However, the development of IB in Sinocyclocheilus cavefish is still unknown. It is critical to reveal the important function of IB in cavefish. In this research, our results describe the IB in Sinocyclocheilus cavefish first time, which provide a foundational knowledge for further genetically modification works.
Materials and Methods:

Cavefish Samples

The 110 individuals of *Sinocyclocheilus* which including *Sinocyclocheilus maitianheensis*, *Sinocyclocheilus qiabeinensis*, *Sinocyclocheilus anophthalmus*, *Sinocyclocheilus macroscalus*, *Sinocyclocheilus guishanensis*, *Sinocyclocheilus hageibarbus*, *Sinocyclocheilus malacopterus*, *Sinocyclocheilus lateristritus*, *Sinocyclocheilus angustiporus*, *Sinocyclocheilus yangzongensis*, *Sinocyclocheilus grahami*, *Sinocyclocheilus xichouensis*, *Sinocyclocheilus tingi*, *Sinocyclocheilus macrolepis*, *Sinocyclocheilus brevis*, *Sinocyclocheilus multipunctatus*, *Sinocyclocheilus furcodorsalis*, *Sinocyclocheilus anatrirostris*, *Sinocyclocheilus rhinocerous*, and *Sinocyclocheilus jii* were screened by X-ray imaging. The sample size and collect data of each species were listed in table 1. Among them, 39 individuals were collected from field locate at Xichou County, Wenshan City, Yunnan Province, and the others were preserved in Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences.

Table 1, the information about the fish used in research.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample size</th>
<th>Collect data</th>
<th>Range of IB count</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. maitianheensis</em></td>
<td>3</td>
<td>2008</td>
<td>102-110</td>
</tr>
<tr>
<td><em>S. qiabeinensis</em></td>
<td>6</td>
<td>2005/2008</td>
<td>107-115</td>
</tr>
<tr>
<td><em>S. anophthalmus</em></td>
<td>3</td>
<td>1987</td>
<td>108-113</td>
</tr>
<tr>
<td><em>S. macroscalus</em></td>
<td>4</td>
<td>2004</td>
<td>108-113</td>
</tr>
<tr>
<td><em>S. guishanensis</em></td>
<td>2</td>
<td>2013</td>
<td>114-120</td>
</tr>
<tr>
<td><em>S. hageibarbus</em></td>
<td>9</td>
<td>2005</td>
<td>101-112</td>
</tr>
<tr>
<td><em>S. malacopterus</em></td>
<td>3</td>
<td>1977</td>
<td>105-118</td>
</tr>
<tr>
<td><em>S. lateristritus</em></td>
<td>5</td>
<td>2004</td>
<td>113-117</td>
</tr>
<tr>
<td><em>S. angustiporus</em></td>
<td>3</td>
<td>2008</td>
<td>111-117</td>
</tr>
<tr>
<td><em>S. yangzongensis</em></td>
<td>3</td>
<td>1963</td>
<td>106-113</td>
</tr>
<tr>
<td><em>S. grahami</em></td>
<td>36</td>
<td>2010/2014</td>
<td>100-114</td>
</tr>
<tr>
<td><em>S. xichouensis</em></td>
<td>2</td>
<td>2013/2014</td>
<td>113-114</td>
</tr>
<tr>
<td><em>S. tingi</em></td>
<td>8</td>
<td>1983/2014</td>
<td>104-112</td>
</tr>
<tr>
<td><em>S. macrolepis</em></td>
<td>3</td>
<td>2004/2008</td>
<td>106-108</td>
</tr>
<tr>
<td><em>S. brevis</em></td>
<td>4</td>
<td>2008</td>
<td>111-114</td>
</tr>
<tr>
<td><em>S. multipunctatus</em></td>
<td>1</td>
<td>2005</td>
<td>104</td>
</tr>
<tr>
<td><em>S. furcodorsalis</em></td>
<td>3</td>
<td>2008</td>
<td>100-102</td>
</tr>
<tr>
<td><em>S. anatrirostris</em></td>
<td>4</td>
<td>2013</td>
<td>84-90</td>
</tr>
<tr>
<td><em>S. rhinocerous</em></td>
<td>3</td>
<td>2012</td>
<td>92-100</td>
</tr>
</tbody>
</table>

X-ray screening

X-ray diagram used in this research is Digital Cabinet X-ray System (Xpearl 80, Kubtec, 270 Rowe Avenue, Unit E Milford, CT 06461, USA). Choose the corresponding electricity and voltage based on different size. According to the insert location of basipterygium, fish body were divided into three regions: before the first basipterygium of dorsal fin (BFBD), between the first basipterygium of dorsal fin and the first basipterygium of anal fin (BFDA), after the first basipterygium of anal fin (AFBA).

Dissection

The *S.grahami* is chosen as a primary example to research the IB in sections. The frozen fish were thawed out and parcelled in silver paper, then boiled and cooled down. Fish body flat on the dissecting tray, and used forceps to pull out the IB from the head muscle to tail muscle. Intermuscular bones stick on the scotch tape in pull out order, and photographed them by digital camera.

Principal component analysis of *Sinocyclocheilus* fishes

Principal component analysis was made to analyze the quantitative relationship of different species in *Sinocyclocheilus* fishes. Variables included total numbers of IB, epineural, epipleural, the intermuscular bone counting before the first basipterygium of dorsal fin, between the first basipterygium of dorsal fin and the first basipterygium of anal fin and after the first basipterygium of anal fin.

Data analysis

The Adobe Photoshop CS4 was used to differentiate categories of IB, and the counter software was used to account the number of IB. The SPSS 19.0 was used for further principal component analysis.

Figure 1. Different forms of intermuscular bones in *Sinocyclocheilus*. non-forked type (A); one-end-unequal-bi-fork type(B); one-end-equal-bi-fork type(C); one-end-multi-fork type(D); two-end-bi-fork type(E); two-end-multi-fork type(F). Scale bars=1mm.
Results:

Different individuals of same species have different number of intermuscular bones

Based on the X-ray diagrams of the 110 individuals (Table 1), the IB counting in Sinocyclocheilus fishes fell into the range 84-120, epineurals fell into the range 51-78, and epipleurals fell into the range 32-44. According to the opinion from Zhao (2008), there are four clades of Sinocyclocheilus fishes, namely clade jii, clade angularis, clade cyphotergous, and clade tingi. The IB counting of these four clades was shown in the Table 2. Different individual of same species do not have same IB count. In addition, the IB number of fish body in the left and right sides are not the same.

Morphotype and attachment site

Through dissected 33 samples of Sinocyclocheilus grahami, they possessed six forms of IB, including non-forked, one-end-unequal-bi-fork, one-end-equal-bi-fork, one-end-multi-fork, two-end-bi-fork, two-end-multi-fork (Figure 1). The anatomical studies of S. tingi and S. xichouensis have same results as above, and screened by X-ray image, there is no more complex form in Sinocyclocheilus fishes. Results showed (Table 2) that the IBs in Sinocyclocheilus fishes are divided into two categories, epineural and epipleural. The opening direction of epineural and epipleural are from tail to head mostly, but on the base of tail, there have few intermuscular bones which opening direction is toward tail.

Principal component analysis of intermuscular bone counting in Sinocyclocheilus fishes

Principal component analysis of six variables showed that the two principal components accounted for 85.222% accumulation contribution rate. Among this 20 kind species, six many species are troglobite fishes, them are S. hughelbarus, S.anophthalmus, S.furcodorsalis, S.anatrirostris, S.rhinoceros, S. multipunctatus, and the other species are troglophile fishes. Based on the results of scatter plot (Figure 2) of the two principal components, the results showed that three troglobite species deviated from most individuals, such as S.anatrirostris, S.rhinoceros and S. furcodorsalis. However, other three troglophile species including S. anophthalmus, S.multipunctatus and S. hughelbarus are not deviated.

Table 2, the IB quantities distributions in Sinocyclocheilus.

<table>
<thead>
<tr>
<th>Sinocyclocheilus fishes</th>
<th>TN</th>
<th>BFBD</th>
<th>AFBA</th>
<th>BFDA</th>
<th>EN</th>
<th>EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>84–120</td>
<td>4–18</td>
<td>52–72</td>
<td>27–39</td>
<td>51–78</td>
<td>32–44</td>
<td></td>
</tr>
<tr>
<td>clade tingi</td>
<td>100–120</td>
<td>8–18</td>
<td>57–72</td>
<td>28–39</td>
<td>66–78</td>
<td>33–44</td>
</tr>
<tr>
<td>clade jii</td>
<td>106–107</td>
<td>12–16</td>
<td>57–61</td>
<td>32–33</td>
<td>68–71</td>
<td>36–38</td>
</tr>
</tbody>
</table>

BFBD: before the first basipterygium of dorsal fin; BFDA: between the first basipterygium of dorsal fin and the first basipterygium of anal fin; AFBA: after the first basipterygium of anal fin; TN: total number of intermuscular bones; EN: epineural; EP: epipleural.

Discussion:

Sinocyclocheilus is a richest genus of Chinese Cyprinidae fishes in terms of species diversity (Zhao & Zhang, 2006), which received a lot of attention from cavefish research scientist in recent years due to their specific habitats and varied phenotypes (Zhang et al., 2003; Zhang & Dai, 2010; Wilkens, 2010; Ma et al., 2008). Our present study provides a foundation for both research of intermuscular bones in Sinocyclocheilus fishes and breed a new fish species which have few intermuscular bones or not. In subsequent research of cave fishes, this work might be a useful information for research cavefish.

In this study, we found that Sinocyclocheilus fishes have two categories, epineural and epipleural. This is different from what previously published by Dong (2006). The number of IB in Sinocyclocheilus fishes is 84 to 120, smaller than the Cyprinidae fishes whose number is from 99 to 133 (Lv et al., 2007), the S. grahami, S.tangi and S. xichouensis have six forms of IB totally, however they all don’t have complex tree-branch forms, and there is no more complex form in all checked Sinocyclocheilus individuals. This result showed that the evolution of IB in Sinocyclocheilus fish proceed slowly. A possible hypothesis might be that the evolution of morphology of IB is from simple forms to various complicated forms.

Figure 2. The division of attach site of fish IB on radiograph. From snout to the base of caudal fin, radiograph of fish divided three regions, from the left to right is, before the first basipterygium of dorsal fin (BFBA); BFDA: between the first basipterygium of dorsal fin and the first basipterygium of anal fin (BFDA); after the first basipterygium of anal fin (AFBA).

Figure 3. Scatter plot of two principal components in the analysis of intermuscular bone count of Sinocyclocheilus fishes. Troglobites species indicated with different colors from troglophile species which color is red. Among troglophile species, calde angularis indicated with different shape.

Another possible hypothesis is, some species at higher evolutionary level will combine several bones together to make their function more efficient. It might means that the species Sinocyclocheilus locate at a higher
evolutional levels, and correctness of this viewpoint will be demonstrate in the follow-up researchs.

Sinocyclocheilus is a special cyprinid genus that is troglobites with typical cave adaptive characteristics such as lost of pigment and eyes (Zhao & Zhang, 2009). Several species have been studied in detail such as S. anatrirostris, S. rhinocerus, S. furcodoralsis, S. anophthalmus, S. hugeiibarbus and S. multipunctatus (Romero et al., 2009). From principal component analysis results, troglobites fishes are not gather together in the graph, but S. anophthalmus, S. multipunctatus and S. hugeiibarbus are stay close with troglophile species. This six species are troglobites species, but the IB counting of S. anophthalmus, S. multipunctatus and S. hugeiibarbus were adapt cave life subsequently compare to the other three species, and the time of living in cave are shorter than others. So far the researchers still do not come to a conclusion about IB’ function. Here, based on our result, we think limited movement might be a reason. Because the cavefish Sinocyclocheilus live in a dark environment with limited food supplies (Zhang & Dai, 2010), the fish will decrease their movement in order to save the energy. During the long evolution process, the fish with low level of IB development will not affect their fitness in the harsh environment. All these results indicate that the decrease the inter-muscular bones number provide a potential benefit for cavefish adapt the cave environment better.

Competing interests

The authors declare no conflict of interest.

Acknowledgements

We would like to thank Du LN for the help of sample selection, and Min R for her generous guidance and support. This study was supported by grants from the Basic Research Programme of Yunnan Province (2012FB183) to X. F. Pan; the Yunnan Biodiversity Protection Program, a major program of the Chinese Academy of Sciences (Y2006B1181), the Yunnan Province Science and Technology Program (2012CA014), the World Bank Dianchi Project (GEF-MSP grant No. TF051795), the Foundation of Development and Reformation Department of Yunnan Provinceto J.X.Yang.

Received: June 25, 2015

Revised: July 2, 2015

Accepted: July5, 2015

Published: July7, 2015

References


