

**Supplementary Table S1. Codes, sampling localities, haplotypes, and accession numbers of *Huigobio* species and outgroup for molecular analyses. \* Haplotypes downloaded from GenBank**

| Code         | Species                                | Locality   | Drainage                                  | Haplotype                         | Accession no.                  | Source                   |
|--------------|--|--|---|-----------------------------------|--------------------------------|--------------------------|
| ASIZB 218745 | <i>Huigobio heterocheilus</i> sp. nov. |  |   | H1                                | OL321605                       | This study               |
| ASIZB 218746 | <i>Huigobio heterocheilus</i> sp. nov. | Fish market in Guanyang County, Guangxi Zhuang Autonomous Region | R. Xiangjiang, middle Yangtze River Basin | H2                                | OL321606                       | This study               |
| ASIZB 218747 | <i>Huigobio heterocheilus</i> sp. nov. |  |   | H2                                | OL321607                       | This study               |
| ASIZB 218748 | <i>Huigobio heterocheilus</i> sp. nov. |  |   | H2                                | OL321608                       | This study               |
| ASIZB 218750 | <i>Huigobio heterocheilus</i> sp. nov. |  |   | H2                                | OL321609                       | This study               |
| ASIZB 218816 | <i>Huigobio heterocheilus</i> sp. nov. |  |   | H2                                | OL321618                       | This study               |
| ASIZB 218785 | <i>Huigobio exilicauda</i>             |  |   |                                   |                                | H3                       |
| ASIZB 218786 | <i>Huigobio exilicauda</i>             | Shixing County, Guangdong Province                               | R. Beijiang, Pearl River Basin            | H3                                | OL321611                       | This study               |
| ASIZB 218787 | <i>Huigobio exilicauda</i>             |  |   | H3                                | OL321612                       | This study               |
| ASIZB 218788 | <i>Huigobio exilicauda</i>             |  |   | H4                                | OL321613                       | This study               |
| HE-LianZ     | <i>Huigobio exilicauda</i>             |  |   | Lianzhou City, Guangdong Province | R. Beijiang, Pearl River Basin | H5*                      |
| ASIZB 218811 | <i>Huigobio chenhsienensis</i>         | Shengzhou City, Zhejiang Province                                | Cao'ejiang River Basin                    | H6                                | OL321615                       | This study               |
| ASIZB 218812 | <i>Huigobio chenhsienensis</i>         |  |   | H7                                | OL321616                       | This study               |
| ASIZB 218813 | <i>Huigobio chenhsienensis</i>         |  |   | H8                                | OL321617                       | This study               |
| HC-NG        | <i>Huigobio chenhsienensis</i>         | Ningguo City, Anhui Province                                     | Lower Yangtze River Basin                 | H9*                               | KX078810                       | He <i>et al.</i> 2017    |
| HC-HuangS    | <i>Huigobio chenhsienensis</i>         | Huangshan City, Anhui Province                                   | Qiantangjiang River Basin                 | H9*                               | KX078811                       | He <i>et al.</i> 2017    |
| MCHOJ1       | <i>Huigobio chenhsienensis</i>         | Yantou Township, Zhejiang Province                               | Oujiang River Basin                       | H10*                              | KT075097                       | Huang <i>et al.</i> 2016 |
| MCHOJ2       | <i>Huigobio chenhsienensis</i>         | Yongjia County, Zhejiang   | Oujiang River Basin                       | H11*                              | KT075098                       | Huang <i>et al.</i> 2016 |

|                   |                                      |  |  |      |          |                             |
|-------------------|--------------------------------------|--|--|------|----------|-----------------------------|
| FDZM-HCND20111101 | <i>Huigobio chenhsienensis</i>       | Province<br>Ningdu County, Jiangxi                             | R. Ganjiang, middle<br>Yangtze River Basin   | H12* | NC032291 | Li <i>et al.</i> 2018       |
| ----              | <i>Microphysogobio zhangii</i>       | Quanzhou County market,<br>Guangxi Zhuang<br>Autonomous Region | R. Xiangjiang, middle<br>Yangtze River Basin | H13* | KU356196 | Huang <i>et al.</i><br>2017 |
| MFUMJ1            | <i>Microphysogobio fukiensis</i>     | Shaowu City market,<br>Fujian Province                         | R. Futunxi, Minjiang<br>River Basin          | H14* | KM999927 | Huang <i>et al.</i> 2016    |
| MELQZ1            | <i>Microphysogobio elongatus</i>     | Quanzhou County market,<br>Guangxi Zhuang<br>Autonomous Region | R. Xiangjiang, middle<br>Yangtze River Basin | H15* | KU356199 | Huang <i>et al.</i><br>2017 |
| ----              | <i>Microphysogobio tungtingensis</i> | Unknown  | Yangtze River Basin                          | H16* | AY953013 | Yang <i>et al.</i> 2006     |
| ----              | <i>Platysmacheilus exiguus</i>       | Unknown  | Pearl River Basin                            | H17* | AY953015 | Yang <i>et al.</i> 2006     |
| ASIZB 218802      | <i>Platysmacheilus exiguus</i>       | Chaling County, Hunan<br>Province                              | R. Mishui, middle<br>Yangtze River Basin     | H18  | OL321614 | This study                  |
| PL-MC             | <i>Platysmacheilus longibarbatus</i> | Macheng City, Hubei<br>Province                                | Middle Yangtze River<br>Basin                | H19* | KX078815 | He <i>et al.</i> 2017       |
| PL-ND             | <i>Platysmacheilus longibarbatus</i> | Ningdu County, Jiangxi<br>Province                             | R. Ganjiang, middle<br>Yangtze River Basin   | H20* | KX078816 | He <i>et al.</i> 2017       |
| <b>Outgroup</b>   |                                      |  |  |      |          |                             |
| PG-YS             | <i>Pseudogobio guilinensis</i>       | Yangshuo County,<br>Guangxi Zhuang<br>Autonomous Region        | R. Lijiang, Pearl River<br>Basin             | H21* | KX096699 | He <i>et al.</i> 2017       |
| ----              | <i>Squalidus argentatus</i>          | Unknown  | Yangtze River Basin                          | H22* | AY952985 | Yang <i>et al.</i><br>2006  |

**Supplementary Table S2.** Genetic distances of the *cyt b* gene computed by MEGA 6.0. amongst 9 analyzed species of *Huigobio*, *Microphysogobio* and *Platysmacheilus*.

| Species                                  | Intraspecific | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     |
|--|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 <i>Huigobio heterocheilus</i> sp. nov. | 0.002         |       |       |       |       |       |       |       |       |       |
| 2 <i>Huigobio exilicauda</i>             | 0.015         | 0.132 |       |       |       |       |       |       |       |       |
| 3 <i>Huigobio chenhsienensis</i>         | 0.045         | 0.151 | 0.165 |       |       |       |       |       |       |       |
| 4 <i>Microphysogobio zhangii</i>         | n/c           | 0.143 | 0.173 | 0.130 |       |       |       |       |       |       |
| 5 <i>Microphysogobio fukiensis</i>       | n/c           | 0.156 | 0.175 | 0.135 | 0.103 |       |       |       |       |       |
| 6 <i>Microphysogobio tungtingensis</i>   | n/c           | 0.156 | 0.172 | 0.131 | 0.100 | 0.016 |       |       |       |       |
| 7 <i>Microphysogobio elongatus</i>       | n/c           | 0.154 | 0.173 | 0.133 | 0.103 | 0.015 | 0.006 |       |       |       |
| 8 <i>Platysmacheilus exiguus</i>         | 0.008         | 0.161 | 0.184 | 0.151 | 0.128 | 0.134 | 0.126 | 0.128 |       |       |
| 9 <i>Platysmacheilus longibarbatus</i>   | 0.015         | 0.143 | 0.172 | 0.137 | 0.128 | 0.131 | 0.125 | 0.126 | 0.095 |       |
| <b>Outgroup</b>                          | 0.183         | 0.188 | 0.212 | 0.183 | 0.173 | 0.157 | 0.151 | 0.154 | 0.173 | 0.168 |