

Population Diversity and Phylogeography of *Liniparhomaloptera disparis* (Cypriniformes: Homalopteridae) in Coastal Western South China Based on mtDNA Sequences

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## 1. Introduction

South China commonly refers to the tropical and subtropical areas of Guangxi, Guangdong, and Fujian Provinces, plus the islands of Taiwan, Hainan, and the territory of Hong Kong, all near or south of the Tropic of Cancer. Western South China is drained by many rivers including the Pearl and its tributaries, second largest river in China in terms of annual runoff or third in terms of length, and several other rivers that empty into the sea independently. These rivers are not only in proximity to each other geographically but also were closely related historically (Yap, 2002). Since Pliocene of the late Tertiary, transgression and regression of the worldwide Quaternary glacial cycles caused the level of the South China Sea to fluctuate greatly. At glacial maxima most of the continental shelf now beneath the South China Sea was above water, as it was in Oligocene of Tertiary. The coastline of South China during the low sea levels was south of the present Hainan Island (Liu et al., 2002; Liu et al., 2003), the second largest island of China after Taiwan. Many rivers of coastal western South China might have been confluent on the present continental shelf, when multiple streams flowed onto the exposed plain and may have merged with one another (Lei et al., 2008). These potential confluences may have left genetic 'footprints' in the genomes of some freshwater fish populations. Our study searches comprehensively for molecular evidence of the phylogenetic and biogeographical relationships of *Liniparhomaloptera disparis* (Cypriniformes: Homalopteridae) among river systems in western South China including Hainan Island and the coastal mainland from western Guangdong to eastern Guangxi (Fig. 1).

There are three major drainages of Hainan Island; all flow independently into the sea but in different directions with one on the western and two on the eastern sides of the Wuzhi (meaning five fingers) mountain range (Fig. 1). In the Tertiary these rivers became independent as a result of the tectonic uplift of mountains in central Hainan (Zeng et al., 1989). This geological process may have isolated freshwater fish in these rivers, resulting in some genetic differentiation. On the other hand, the area ranging from Beibu Gulf to Qiongzong Strait (Fig. 1) is neritic (under shallow sea water periodically) and was exposed with a fall in sea level of 50-130 m below the interglacial present (Xu et al., 2009). Therefore, the drainages on this area might have been confluent with the drainages of Hainan Island during glacial maxima (Lin, 1985; Zhao et al., 2001). This is supported by relatively close biogeographical relationships among rivers on Hainan Island and their distant relationship to the Pearl River based on freshwater fish faunal similarity in kind (Du et al., 2003), morphometrics and molecular genetics of *Pelteobagrus intermedius* (Siluriformes: Bagridae) (Qing et al., 2007), and molecular analysis of

*Micronoemacheilus pulcher* (Cypriniformes: Cobitidae) (Qiu et al., 2008). The latter two studies both postulated that the dispersal centers of *P. intermedius* and *M. pulcher* were similarly at the confluence of three rivers in eastern Guangxi near northern Vietnam and at the ancient river systems between Hainan Island and Vietnam. They suggested replicate dispersal routes, one from the mainland center to the more peripheral mainland rivers, the other route from the now-underwater center to Hainan first and then back to the mainland, and through these routes, both fish species were expanding northward to western Guangdong and eastern Guangxi. However, samples from the only major river on western Hainan Island were absent in both studies. Lei and He (2008) reported that the upstream populations of *Hemibagrus guttatus* (Siluriformes: Bagridae) in three main tributaries of the Pearl River were closely related, suggesting that was a result of gene exchange during a glacial maximum when cooler upland conditions occurred farther downstream where the tributaries became confluent. Nevertheless, few explored on a larger scale whether freshwater fishes on Hainan Island are differentiated and how they are related to those on the coastal mainland.

*Liniparhomaloptera disparis* (Cypriniformes: Homalopteridae) is a primary freshwater fish. It is small, lives in clear, swift streams that usually have gravel bottoms, and is endemic to western South China including Hainan, ranging peripherally from northern Vietnam to Guizhou, Hunan, Jiangxi, and Yunnan Provinces (Yue, 2000; Tang and Chen, 2000; Tang et al., 2000). Distribution of *L. disparis* in the relatively isolated upper reaches of rivers is conducive to genetic differentiation of populations that can be correlated with paleogeography. Furthermore, morphological differences warrant two subspecies: *L. d. disparis* on the coastal mainland of western South China and *L. d. qiongzhongensis* on Hainan (Zheng and Chen, 1980). Genetic differences were also found within the Hainan populations and among drainages of the coastal mainland from western Guangdong to eastern Guangxi, preliminarily by the mitochondrial DNA (mtDNA) control region with 477 base pairs (bp) and a sample of 87 individuals (Zuo et al., 2009). Both studies suggested that the mountain range on Hainan may have formed an effective geographic barrier facilitating island population differentiation, but the latter study proposed further that the major Yunkai-Yunwu mountain ranges (meaning cloud dispersing and cloud misting, respectively) in western South China acted similarly resulting in mainland population differentiation. The mainland mountain ranges merge near the border between western Guangdong and eastern Guangxi and are as old as those on Hainan Island (Zhao et al., 2001). In addition, because of its presence in most rivers of western South China today that empty into the sea independently, the genetic structure of *L. disparis* might have been affected dramatically by the fall and rise of Pleistocene sea levels. These attributes make *L. disparis* an excellent model for a study of genetic diversity in freshwater fishes and phylogeography of western South China.

In this study we expanded sampling in size, in mtDNA length, and in sites to use 114 individuals, to examine both sequences of the control region and cytochrome b, and to include a broader range for both named subspecies of *L. disparis* and habitats previously not covered in western South China. The objectives of this study are twofold: first, to investigate the phylogenetic relationships and geographical differentiation of *L. disparis* populations throughout most of the distributional range in western South China; second, to correlate the timing of genetic differentiation with geological processes or events such as glacial maxima. Based on current topography and previous work, we divided the *L. disparis* populations into four drainage areas: the Pearl River drainage, the Hainan Island drainages, the eastern mainland independent

drainages, and the western mainland independent drainages (Fig. 1). Rivers of the last two drainage areas on the coastal mainland of western South China are separated from each other by the Yunkai-Yunwu mountain ranges, from the Pearl by other minor mountain ranges, and flow into the sea independently. The rivers have no present connection with either the Pearl or Hainan but may have been connected with those downstream at glacial maxima. We thus split them into the western and eastern mainland using the merging mountain ranges as the divide. Samples were especially sought on either side of the mountain range(s) on Hainan and on the mainland to test if the mountains are effective barriers. We initially hypothesized that the populations of *L. disparis* were differentiated among these four drainage areas where sea water and mainland mountain ranges are barriers that prevent gene flow (isolation by sea level, isolation by mainland tectonics). We then hypothesized that genetic differentiation of Hainan Island populations may have been complicated not only by the separation from the mainland by interglacial sea level rise in Quaternary but also by the mountain lift in Tertiary (isolation by island tectonics). We further hypothesized phylogenetically that the eastern mainland populations of the independent coastal drainages were closely related to those of the Pearl River drainage and the western ones were closely related to some of those on Hainan Island because of the mainland mountain ranges and the island-coastal mainland connection at glacial maxima. Our results not only confirm the validity of *L. d. qiongzhongensis* on Hainan, but also reveal for the first time much higher genetic diversity than expected and close correlation of geographic differentiation of *L. disparis* in western South China with geological events.