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# Species delimitation and phylogenetic reconstruction of the sinipercids (Perciformes: Sinipercidae) based on target enrichment of thousands of nuclear coding sequences

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## ABSTRACT

The sinipercids are freshwater fishes endemic to East Asia, mainly in China. Phylogenetic studies on the sinipercids have made great progress in the last decades, but interspecific relationships and evolutionary history of the sinipercids remain unresolved. Lack of distinctive morphological characters leads to problems in validating of some species, such as *Siniperca loona*. Moreover, genetic data are needed to delimitate species pairs with explicit hypothesis testing, such as in *S. chuatsi* vs. *S. kneri* and *Coreoperca whiteheadi* vs. *C. liui*. Here we reconstructed phylogeny of the sinipercids with an unprecedented scale of data, 16,943 loci of single-copy coding sequence data from nine sinipercid species, eight putative sister taxa and two outgroups. Targeted sequences were collected using gene enrichment and Illumina sequencing, yielding thousands of protein coding sequences and single nucleotide polymorphisms (SNPs) data. Maximum likelihood and coalescent species tree analyses resulted in identical and highly supported trees. We confirmed that the centrarchids are sister to the sinipercids. A monophyletic Sinipercidae with two genera, *Siniperca* and *Coreoperca* was also supported. Different from most previous studies, *S. scherzeri* was found as the most basal taxon to other species of *Siniperca*, which consists of two clades: a clade having *S. roulei* sister to *S. chuatsi* and *S. kneri*, and a clade consisting *S. loona* sister to *S. obscura* and *S. undulata*. We found that both *S. loona* and *C. liui* are valid species using Bayes factor delimitation (BFD*) based on SNPs data. Species delimitation also provided decisive support for *S. chuatsi* and *S. kneri* being two distinct species. We calibrated a chronogram of the sinipercids based on 100 loci and three fossil calibration points using BEAST, and reconstructed ancestral ranges of the sinipercids using Lagrange Analysis (DEC model) and Statistical Dispersal-Vicariance Analysis (S-DIVA) implemented in RASP. Divergence time estimates and ancestral habitat reconstruction suggested a wide-ranging distribution of the common ancestor of the sinipercids in southern China at 53.1 million years ago (CI: 30.4–85.8 Ma). The calibrated time tree is consistent with historical climate changes and geological events that might have shaped the current distribution of the sinipercids.

## 1. Introduction

Sinipercidae (Perciformes) is a group of freshwater fish distributed in China, Korea, Japan, Russia, and Vietnam with most of its species found only in China. The number of valid species in sinipercids is about 9–12 according to different authors (Li, 1991; Liu and Chen, 1994; Nelson, 2006; Zhou et al., 1988). Substantial progress has been made in resolving phylogenetic relationships among the sinipercids. Traditional classification based on morphology agrees on a monophyletic sinipercids (Liu, 1997; Liu and Chen, 1994), which has been erected as a distinct family (Nelson, 2006; Roberts, 1993). Molecular data also generally support the monophyly of the sinipercids (Li et al., 2010; Near et al., 2012; Zhao et al., 2008, 2006a, 2006b), except that Chen et al. (2007) found that the sinipercids were paraphyletic based on cytochrome b (cytb) sequence.

Morphological studies have competing hypotheses about the sister group of the sinipercids, such as the Serranidae (Jordan, 1923; Nichols, 1943; Zhou et al., 1988) and the Percichthyidae (Gosline, 1966), but consistent results have not been reached (e.g., (Chang, 1988; Johnson, 1984; Liu, 1997; Liu and Chen, 1994; McCully, 1962; Nelson, 1994; Waldman, 1986; Zhou et al., 1988). As for molecular studies, Zhao et al. (2005) found a clade consisting of *Perca* and *Pristigenys* as the sister taxon of the sinipercids. Smith and Craig (2007) supported a clade of the percichthyids

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and the centrarchids as the sister taxon of the sinipercids. More recent studies using molecular data recovered a sister-group relationship between the sinipercids and the centrarchids (Chen et al., 2014; Li et al., 2010; Near et al., 2012).

The sinipercids were classified into three genera, *Coreoperca*, *Siniperca* and *Coreosiniperca*, each containing three, seven and one species respectively (Zhou et al., 1988; Zhu, 1985). A cladistic analysis based on 34 morphological characters grouped *Coreosiniperca roulei* with species of *Siniperca* (Liu and Chen, 1994), which was also supported by molecular data (Chen et al., 2010; Li et al., 2010; Zhao et al., 2006a). Although previous molecular studies greatly improved our understanding of the interrelationships among major clades of the Sinipercidae, interspecific relationships among the sinipercids still remain unresolved. Several different molecular phylogenetic analyses aimed at resolving interrelationship among species produced controversial results (Chen et al., 2007, 2010; Li et al., 2010; Zhao et al., 2008, 2006a, 2006b). For example, mitochondrial loci, nuclear loci or combined data supported different relationships among the sinipercids (Fig. 1; (Chen et al., 2010; Li et al., 2010; Zhao et al., 2006a, 2006b). Among those studies, the only congruent result was a confirmed sister relationship between *S. chuatsi* and *S. kneri*, whereas the other relationships were either inconsistent or having low support (Fig. 1).

More important problem challenging systematics of the sinipercids is that the species limits are still unclear. For example, *S. loona* is often considered as a synonym of *S. obscura* (Zhou et al., 1988), but Liu and Chen (1994) argued that *S. loona* should be a valid species based on a couple of morphological characters and its allopatric distribution from *S. obscura* (Fig. S1). Cryptic species was also found in *Coreoperca.* The holotype of *Coreoperca whiteheadi* Boulenger, 1900 was found in the Nandujiang River, Hainan, Chinese southernmost island province. Cao et al. (2013) described a new species, *Coreoperca liui*, on the basis of 19 specimens collected from the Qiantangjiang River basin in Zhejiang province, southeastern China (Fig. S2). The newly described species was similar to *C. whiteheadi* morphologically, only distinguishable in a couple of morphometric characters, but no genetic data were collected to verify the distinctness of the new species. Finally, *S. chuatsi* and *S. kneri* are partially sympatric (Fig. S3), and they have obviously morphological differences in characters, such as number of plyoric-caecum, ratio between eye length and head length (Zhou et al., 1988), but current molecular data (mitochondrial control region) could not separate them as different species (Zhao et al., 2008).

The sinipercids are endemic to East Asia, widely distributed in most Chinese river drainages, so it could represent an interesting group for biogeographic studies on Chinese freshwater fish fauna. Delimiting species boundary and resolving the species phylogeny of the sinipercids are the first step to understand the origin of the sinipercids, the impact of past geological events on their evolutionary patterns, and the evolution of Chinese freshwater fish fauna in general.

A great number of independent loci could result in better-resolved phylogenies (Corl and Ellegren, 2013; Kimball and Braun, 2014). In this study, we applied a targeted gene capture method (Li et al., 2013) to generate a large amount of protein coding sequences and single nucleotide polymorphisms (SNPs) data from eleven species (33 individuals) of the sinipercids, as well as 10 outgroup taxa. Our objectives are: (1) to delimit species status between *C. liui* and *C. whiteheadi*, *S. obscura* and *S. loona*, and *S. chuatsi* and *S. kneri* using multiple independent nuclear loci; (2) to resolve the undetermined interspecific relationships within the sinipercids; (3) and to reconstruct the evolution history of the sinipercids using fossil calibration, with regard to species divergence time and ancestral area reconstruction. Our work may provide a backbone for a better understanding of evolutionary history, biogeography and conservation of the sinipercids.

## 2. Materials and methods

### 2.1. Taxon sampling and DNA extraction

Thirty-three individuals from nine species of the sinipercids were sampled and each species was represented by two to ten individuals. The sampling covers all species of the family except for *S. fortis*, *S. liuzhouensis* and *S. robusta* due to availability (Table 1). Ten outgroup species were examined, including *Micropterus salmoides*, *Pomoxis nigromaculatus* (Centrarchidae), *Lateolabrax*

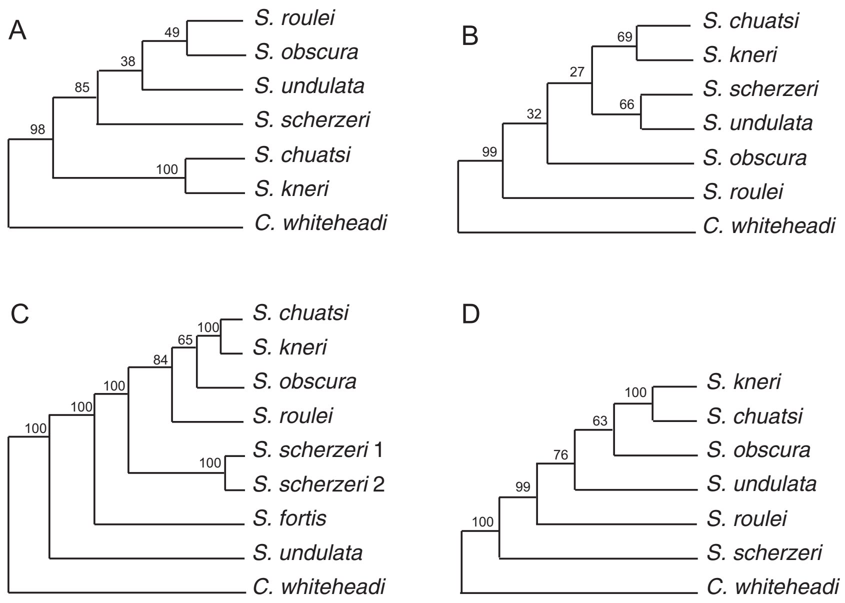

**Fig. 1.** Different hypotheses about the interrelationship among the sinipercids proposed by previous studies: (A) MP tree based on mitochondrial DNA control region (Zhao et al., 2006b); (B) MP tree obtained from cytochrome *b* sequence (Zhao et al., 2006a); (C) ML phylogeny inferred from combined data including mtDNA, nuclear loci, and viperin (Chen et al., 2010); (D) species tree based on 11 nuclear loci (Li et al., 2010).