

Molecular phylogenetic relationships of the southern Africa endemic gobies of the genus *Caffrogobius* Smith, 1900 (Teleostei, Gobiidae)

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Background: The genus *Caffrogobius* consists of seven nominal Western Indian Ocean species, six of which (*C. agulhensis*, *C. caffer*, *C. gilchristi*, *C. natalensis*, *C. nudiceps* and *C. saldanha*) occur in the southern Africa region (Mozambique to Namibia). There's a high degree of morphological similarity within the genus such that the identification of species using existing dichotomous keys posed a challenge. The present study aims to review the relationship among the six southern African *Caffrogobius* species using molecular analyses..

Methods: Specimens were collected in estuaries/rivers and marine tide pools in South Africa with the total number about 45 sites. DNA sequence data sets were generated for a fragment of two mitochondrial regions, cytochrome b (*cyt b*) and cytochrome c oxidase I (COI) genes. These sequences were analysed to estimate divergence distance between species and construct a maximum likelihood phylogenetic tree to assess the relationship among these species.

Results: The phylogenetic tree showed a monophyletic genus *Caffrogobius* with five strongly supported main clades representing the six species. Four of these clades represented *C. caffer*, *C. natalensis*, *C. nudiceps* and *C. gilchristi* and the fifth main clade represented *C. agulhensis* and *C. saldanha*. The sequence divergence between the first four species was high enough to suggest that these are different species. Although *C. agulhensis* and *C. saldanha* were grouped in separate subclades, the genetic distance between them was very low. This suggests that this group might have evolved within a relatively short time period and have not yet been sorted into unique lineages. It is, however, also possible that our identifications of *C. agulhensis* were incorrect and this species has not been collected in any of our sampling activities so far. *Caffrogobius natalensis*, an estuarine species that is restricted to the warm-temperate region of southern Africa, was basal in the genus being sister to all the other taxa. This suggests that the last common ancestor of the genus probably occupied estuarine systems, subsequently diverging into several species adapted to new habitats in the coastal and marine environment.

Conclusion: The sister group relationships between euryhaline and marine species and the absence of a single origin for the marine species support a scenario of repeated events of isolation and invasion of the two habitats probably due to sea-level changes.