

**Comparative genetic and morphometric
characterization of sympatric populations of
Heterobranchus bidorsalis and *H. longifilis***

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INTRODUCTION

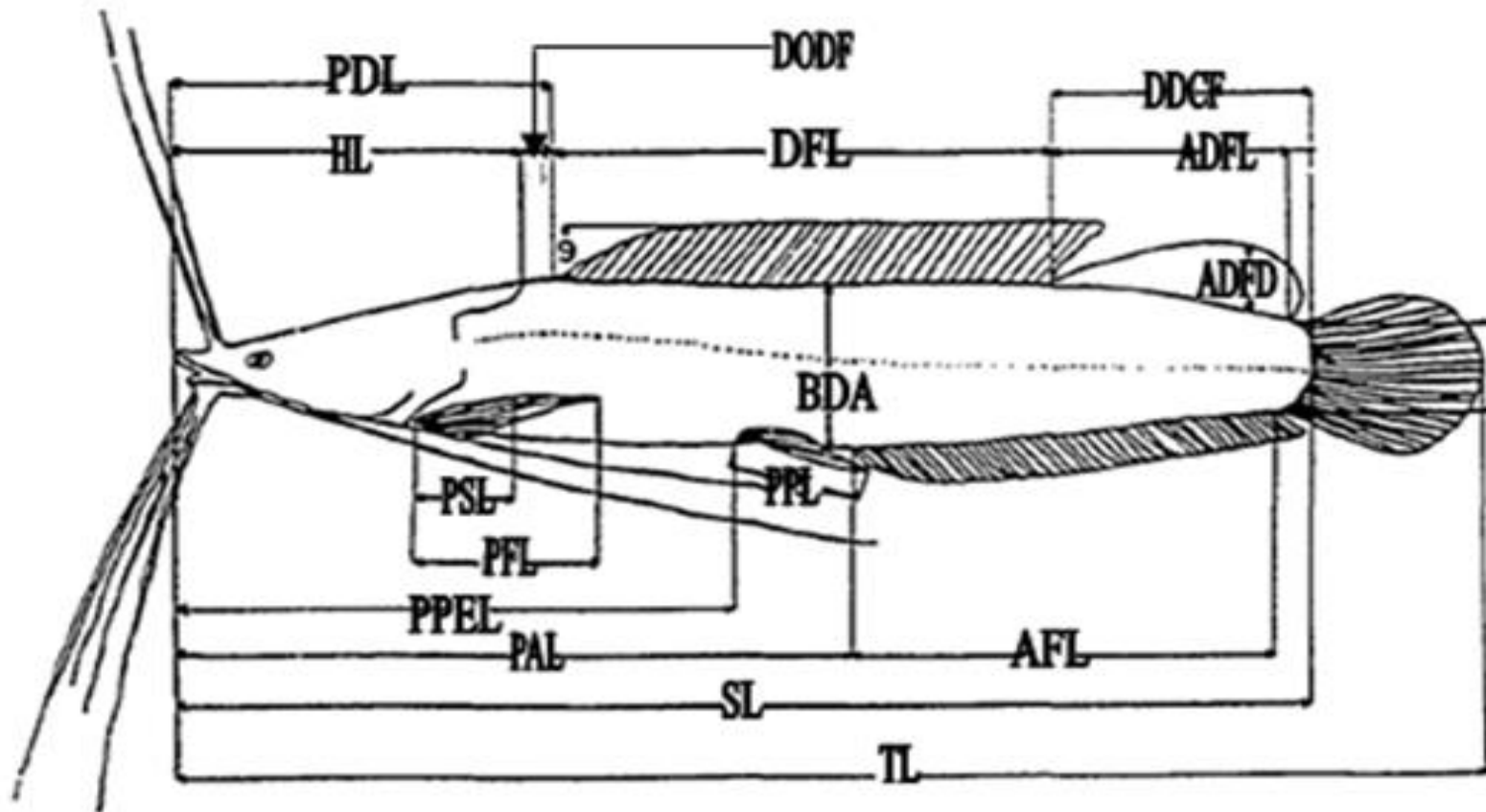
- *Heterobranchus bidorsalis* Geoffroy Saint Hilaire, 1809 and *H. longifilis* Valenciennes, 1840 (Teugels et al., 1990).



- The present study has employed molecular analysis using blood serum protein in addition to morphometry for characterization of the sympatric *Heterobranchus* species

Materials and Methods

- **Serum preparation:** 0.9% NaCl was added at 3:2 blood samples; and left at ambient temperature for 1 h; centrifuged at 3,000 rpm for 10 min. The supernatant was stored at -20 °C for further analysis (Avtalion, 1984; Betiku and Omitogun, 2006).
- **Gel preparation:** SDS-PAGE gel was carried out using the Bio-Rad Mini Protean II Cell kit of 10 ml capacity. A discontinuous buffer system analysis was employed. Solutions for 4% stacking gel, 12% resolving gel was prepared (Bio-Rad, 1995).
- **Sample preparation for SDS-PAGE:** 30-40 μ l of 7.5 % β -mercaptoethanol was added to 370 μ l of sample buffer. Each of 10 μ l protein sample, 40 - 60 μ l of mixture of sample buffer plus β -mercaptoethanol was added at ratio 1:5. Prepared samples were heated at 95 °C for 4 min for denaturation. Thereafter 10 μ l each was loaded.
- **Staining and de-staining:** After the electrophoretic run, the gels were stained in 0.1% Coomassie blue in glacial acetic 1:4 methanol for ~ 1 h. Thereafter, the gels were destained with 60% glacial acetic 1:4 methanol solution for ~3hrs. The gel was then documented.
- **Data analysis:** Gel was scored for presence (1) or absence (0) of protein bands. Data were log transformed and analysed with PAleontological STatistics (PAST) software to generate dendrograms. Mean value of each species was employed to generate distance indices data for comparative genetic distance evaluation choosing Dice option.



TL, total length; SL, standard length; HL, head length; PAL, pre-anal length; PPL, pre-pelvic length; PPEL, pre-pectoral length; PDL, pre-dorsal length; DFL, dorsal fin length; ADFL, adipose fin length; ADFD, adipose fin depth; AFL, anal fin length; PFL, pelvic fin length; DFR, dorsal fin ray; AFR, anal fin ray

Results and Discussion

Table 1. Measurements and meristic counts for population of *H. bidorsalis* and *H. longifilis*

Parameters	<i>H. bidorsalis</i>			<i>H. longifilis</i>		
	n	mean	SD	N	mean	SD
TL (cm)	32	51.7	4.1	35	53.7	4.6
SL (cm)	32	45.9	3.7	35	47.1	3.9
			%SL			
HL	32	29.9	0.9	35	31.2	1.4
PAL	32	58.4	3.3	35	63.2	4.3
PPL	32	48.0	1.6	35	49.4	2.3
PPEL	32	21.7	1.5	35	23.0	1.3
PDL	32	34.6	1.2	35	38.6	2.2
DFL	32	42.3	1.7	35	35.2	1.7
ADFL	32	23.4	1.1	35	25.7	3.2
ADFD	32	4.2	0.5	35	4.4	0.6
AFL	32	38.7	2.2	35	34.3	2.6
PFL	32	10.5	1.0	35	9.7	1.2
			Fin Rays			
		Min-Max			Min-Max	
DFR	32	40-45		35	26-32	
AFR	32	39-55		35	26-41	

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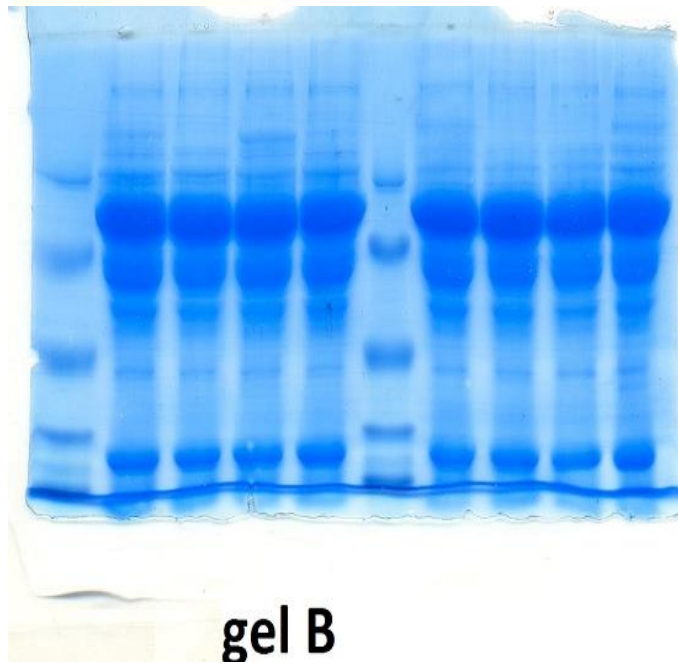
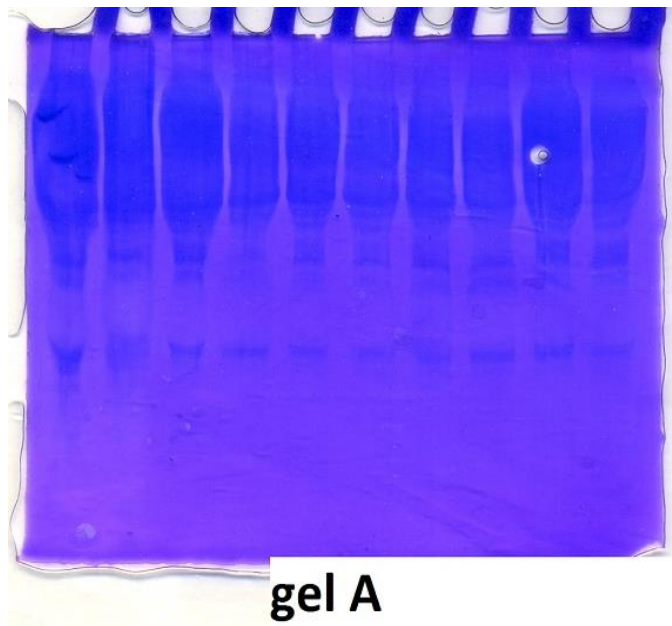


Figure 1. SDS-PAGE representative gels of the samples revealing sera protein bands; gel A, *H. longifilis*; and gel B, *H. bidorsalis*.

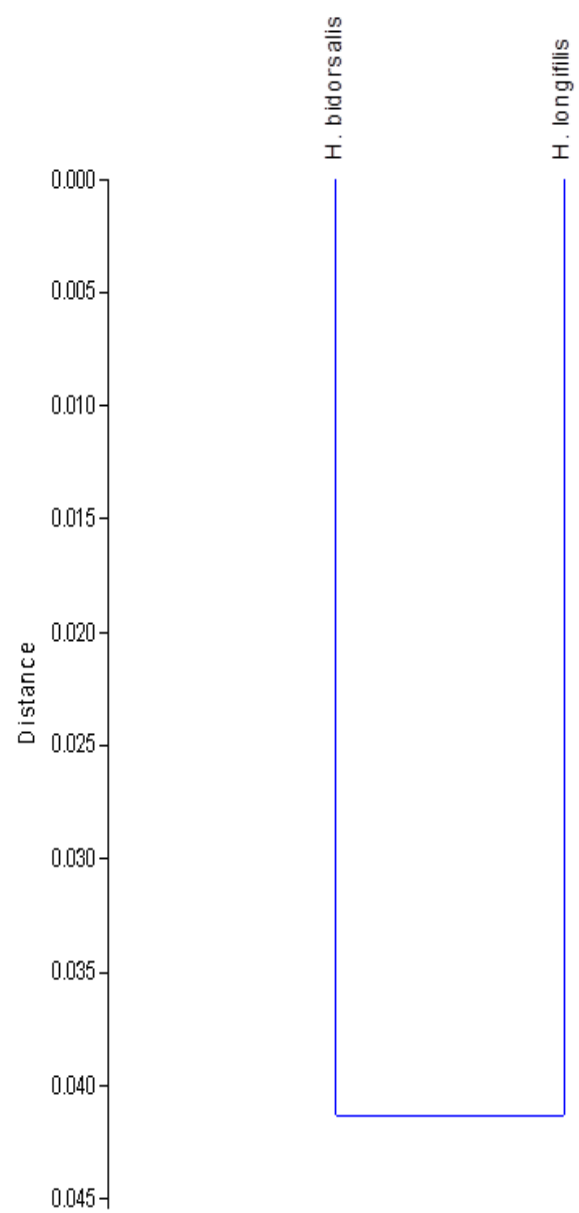


Figure 2. Dendrogram showing genetic relationships between *H. bidorsalis* and *H. longifilis* species

Conclusion

- Both species are closely related genetically but significantly different i.e. not the same. Therefore, they are very close substitutes for each other especially in breeding programs such as hybridization. The hyper-development of the adipose attributes quickly assists in their identification.

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attention*