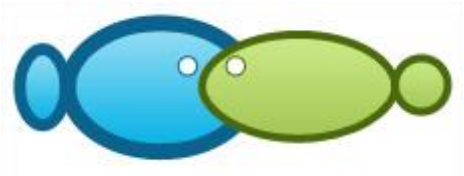

Successful hybridization between *Clarias microstomus*♂ and *Clarias gariepinus*♀

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Abstract. Hybridization is a significant approach to breeding that enables the creation of new varieties that enhance genetic diversity. Through fish inter-species hybridization, it is possible to produce hybrids that can be utilized in aquaculture and stocking programs to enhance growth rate, transfer desirable traits between species, and combine the favourable attributes of two parents into a single progeny. The present study revealed the successful hybridization between *Clarias microstomus* (Ng, 2001) ♂ and *Clarias gariepinus* (Burchell, 1822) ♀ for the first time in the aquaculture industry. *C. microstomus* is a species of Clariid catfish endemic to the island of Borneo and *C. gariepinus* is an introduced species widely aquacultured throughout the southeast Asian region. The embryonic development of the hybrid offspring is described with the hatching percentage of 58.63% and the early survival rate for the first 72 hours for the larvae of 85.76%. The hybrids showed no signs of deformities and developed normally. The findings of the study provide a new high yield variety of catfish for farmers and aquaculture industry for increasing production and profit margin.
Key Words: Clariid catfish, crossbreeding, embryonic development, endemic species, Malaysian Borneo.

Introduction. The most cultured fin-fishes worldwide are tilapia, carp, salmon and catfish (FAO 2021). The culture of African catfish (*Clarias gariepinus*) in Malaysia is a major contributor to Malaysia's aquaculture industry, being the highest single species finfish aquaculture output, contributing with 10% to the nation's total aquaculture production (Dauda et al 2018). *C. gariepinus* is native to Africa and was introduced to Malaysia as an aquaculture species via Thailand in the mid 1980's (Dauda et al 2018). The island of Borneo has a rich diversity of native clariid species (Hee 1999; Ng 2001, 2003). *C. microstomus* is a relatively new species to science. This species is endemic to Borneo and was first described by Heok-Hee Ng in 2001 (Ng 2001). *C. microstomus* belongs to the *C. leiacanthus* species group, as defined by Ng (1999). Species in this group have short bodies with 62-74 dorsal-fin rays. *C. microstomus* differs from all congeners in this group by having a narrower snout and mouth and a deeper body (Ng 2001). *C. microstomus* is currently listed as data deficient under the IUCN Redlist for endangered species (Ng 2001, 2019). In general, all species from the genus *Clarias* are widely favored for aquaculture and as food fish due to their low count of intramuscular bones, good flavor, fast growth rate and ability to be cultured under less than optimum water quality conditions (Goda et al 2007; Zafar et al 2017; Ferosekhan et al 2022). However, the aquaculture of native species of *Clarias* in Borneo has been neglected in favor of African catfish (Jabarsyah et al 2022).

The production of native species for aquaculture is favorable to non-native species due to a number of reasons such as bio security, ecosystem degradation, loss of biodiversity and extinction of native species (Ju et al 2020; Abit et al 2021). Despite the significant economic importance of local *Clarias* species, like *C. microstomus*, their successful aquaculture has faced numerous challenges (Tine 2021; Mbokane et al 2022). These obstacles primarily stem from the scarcity of research conducted on reproductive and culture techniques, as well as the unavailability of suitable brood stock and fries (Adebayo et al 2012; Uedeme-naa & Nwafili 2017). Moreover, when compared to the

non-native *C. gariepinus*, local species exhibit relatively smaller overall size and slower growth rates (Kadye & Booth 2012; Tiogué et al 2020). These factors collectively hinder the development and expansion of aquaculture practices for local *Clarias* species.

Hybridization of local clariids with *C. gariepinus* could be a partial solution to the problem to biosecurity posed by the widespread aquaculture of *C. gariepinus* in Malaysia (Na-Nakorn & Brummett 2009; Haymer & Khedkar 2022). Hybridization involves the procreation between differentiated individuals and involves crossbreeding within a species or between two separate species (Bartley et al 2000; Epifanio & Nielsen 2000; Wachirachaikarn et al 2009; do Prado et al 2017; Pinheiro et al 2019). Hybridization is a technique used by aquaculturists to produce aquatic organisms with the combined desirable traits (i.e. increased growth rate, disease resistance, higher food conversion ratio, increased environmental hardiness, etc.) from two different species, while reducing unwanted reproduction by producing sterile offspring (Rahman et al 2013). The term hybridization as used in the field of aquaculture may refer to intraspecific, interspecific and intergeneric hybridizations (Tiogué et al 2020). Intergeneric and interspecific hybridization of *C. gariepinus* with other related species have been successfully carried out with some of the resultant offspring showing positive heterosis for desirable traits such as survival and growth (Olufeagba et al 2016; Okomoda et al 2017b). In neighboring Thailand, catfish aquaculturists show a far greater preference for hybrid catfish (local Thai catfish *C. macrocephalus* and African catfish *C. gariepinus*) in comparison to pure strains of either species. This can be attributed to the inheritance of desirable qualities in the hybrid fish from both parent fish (Bartley et al 2000). So far, to our best knowledge and based on the published academic documents, no artificial breeding approach was conducted to reproduce *C. microstomus*, while the result of cross breeding of this species with well-studied *C. gariepinus* is completely unknown. The higher fecundity of *C. gariepinus* in comparison to *C. microstomus* allows for a probable larger volume of larvae production. Thus, the present study explored the possibility to create a local hybrid for use in aquaculture and presents the first report on the breeding performance, hatching rate as well as embryonic and early larval development of the offspring of *C. microstomus* (Ng, 2001)♂ cross breed with *C. gariepinus* (Burchell, 1822)♀.

Material and Method

Brood stock management and breeding. Two adult males of *C. microstomus* (mean weight 0.32±0.06 kg) and two adult female *C. gariepinus* (mean weight 0.85±0.04 kg) were obtained from the Department of Animal Science and Fishery, Universiti Putra Malaysia, Bintulu Campus, Malaysia. Male and female ratio was 1:1. The fish were acclimatized for 10 days in two separate rectangular locally made HDPE tanks, with a capacity of 360 L (Figure 1). The brood fish were maintained on commercial floating pelletized starter diet (Cargill), due to its high content of crude protein, 37%. Female broodstock were maintained until their abdomens were observed to be soft and swollen, with their genital papilla becoming pinkish red in coloration, indicating the presence of mature oocytes in the ovary. In a single breeding trial, hybridization was attempted between male *C. microstomus* and female *C. gariepinus*, using two pairs of fish induced to spawn using Ovaprim® (Syndel, USA), a commercially available analogue of Salmon GnRh (1 mL of Ovaprim contains 20 pg of GnRH and 10 mg domperidon). Ovaprim is a popular injectable ovulating/spermiating agent for fin fishes in Malaysia (Marimuthu 2019). Induced breeding was carried out using the standard method for *C. gariepinus* (Abit & Latif 2021), with a dose of 0.5 mL kg^{-1} Ovaprim® per fish for female brood fish and 0.25 mL kg^{-1} Ovaprim® for male brood fish, which was administered intramuscularly into the base of the dorsal fin. Male and female fish were maintained in two separate tanks. After injection, fish were returned to their holding tanks and inspected at 4-hour intervals for signs of spawning. Both fish species had a similar latency period of 12 hours using the aforementioned dosage of Ovaprim®. After 12 hours, eggs from the female *C. gariepinus* fish were stripped into two bowls *C. microstomus* males were tranquilized using a 150 mg L^{-1} solution of methane sulphonate (MS222) (Okomoda et al 2017a)

before being dissected to remove their testes, which were subsequently macerated in a mixing bowl to release sperm. The resulting sperm mixture was used to fertilize the two egg batches obtained prior. Sperm and eggs were mixed together and stirred using a sterile chicken feather for 1 min. A small amount of saline water (50 mL) was added. Excess sperm and water was decanted to leave only the fertilized eggs. The fertilized eggs were poured in a single layer onto floating nylon mesh hatching nets, over continuously aerated water in two 96 L HDPE tanks. Water in the spawning tanks was treated with methylene blue to delay fungal growth, while any protein build up was removed by skimming the surface water with a fine mesh net. A 20% water exchange was carried out every 2 hours by siphoning from the bottom of each tank and slowly replacing the water back to the previous level. Ambient room temperature was kept at a constant 29^{o}C throughout the trial with the use of a room air conditioner.

Figure 1. Adult *Clarias microstomus* male ready for cross breeding with *Clarias gariepinus.*

Embryonic and larval development analysis. Embryonic and larval development of the fertilized eggs were observed under a dissecting magnification device and photographed. A Leica Zoom 2000 (Model, Gxm L3200) and Leica CME microscopes were used to monitor the embryonic and larval development. The progressing development (40 to 100X magnification) of eggs was recorded photographically at 1-hour intervals until hatching occurred. Early larval development was monitored and photographed at 6-hour intervals until the formation of the fish gut. Hatched larvae were maintained without food for the first 72 h before being fed on a regiment of HUFA (Highly Unsaturated Fatty Acids) enriched *Artemia* nauplii.

Measurement of egg diameter and larval length. Egg diameter measurements were conducted on eggs at maturity levels III and IV of gonads, with a total of 50 eggs observed. The observations were made using a microscope (Leica Zoom 2000, Model Gxm L3200, and Leica CME microscopes) equipped with a micrometer. The total length of the larvae was measured with a dissecting microscope (Leica Zoom 2000 Model, Gxm L3200) equipped with an ocular micrometer, with precision up to 0.1 mm.

Data analysis. The hatching percentage was determined by dividing the total number of larvae by the total number of spawned eggs multiplied by 100%. Survival rate of larvae

was calculated as the number of larvae that survived from hatching until the formation of gut at 72 hours multiplied by 100%.

Results and Discussion. The hatching percentage was 58.63% of the total eggs and the early survival rate for the first 72 hours was 85.76%. The mean diameter of the eggs obtained from the *C. gariepinus* brood fish were 0.66±0.02 mm prior to being fertilized and 0.89±0.03 mm after fertilization occurred. The hatching rate for the hybrid cross between *C. microstomus*♂ and *C. gariepinus*♀ was 58.63% in the present study, being lower than that of some other reported hybrids of *C. gariepinus* such as *C. gariepinus* hybridized with *Heterobranchus longifilis*, of 72.26% (Olufeagba et al 2016). However, it was higher than that of some other hybrids such as *C. gariepinus*♀ hybridized with *Pangasiodon hyphothalamus*♂, of 49.04% (Okomoda et al 2017b). The hatching percentage of the hybrid between *C. gariepinus* and *C. microstomus* was found comparatively low in the present study, which might be due to the lack of intensive broodstock management, diet management, and operational management (Hossain et al 2016; Islam et al 2016, 2017; Rahman et al 2016; Shabuj et al 2016; Yeasmin et al 2018). The fertilized eggs were olive brown in color, spherical in shape and adhesive in nature. The different stages of embryonic development are summarized in the following Table 1 and in Figure 2.

The newly hatched larvae were translucent and light brown in color, with an average total length of 2.5±0.03 mm. Each hatchling larvae had a large translucent olive green color yolk sac. Larvae were seen to be free from deformities. The eyes of newly hatched larvae were unpigmented, with fins and mouths not visible at this stage. Within 72 hours, the yolk sac of the majority of larvae had receded as their guts and other primary and secondary features formed and the larvae began feeding. In embryonic development, the larvae went through all the major stages similar to those described for other clariid species such as the giant African catfish, *Heterobranchus bidorsalis* (Olaniyi & Omitogun 2014a) and *C. gariepinus*, which suggested that an 85% hatching rate can be obtained with a high maintenance of broodstock and proper breeding management (Olaniyi & Omitogun 2014b).

A previous study found abnormal deformities in the cleavage stage of early embryonic development in *C. gariepinus*, resulting in a lower survival rate (Hassan et al 2018). Another study of hybridization between *P. hypophthalmus* and *C. gariepinus* observed high unequal cell cleavages leading to different forms of deformities (Okomoda et al 2017b). On the other hand, Borode et al (2002) suggested that salinity might be a key factor of deformity in the early stage embryonic development of *C. gariepinus*, resulting low survival of the hatchlings. However, in our induced breeding process we did not use any saline water. In general, the current study did not found any deformities in the early larval development of the hybrid, resulting in higher survival rates.

The induced breeding of *C. gariepinus* and their early stage embryonic development were observed by many researchers, who tried to work with different hypotheses, general observations, juvenile productions (Sule & Adikwu 2004; Behmene et al 2022). In our study, we maintained a room air temperature of 29°C throughout the hybridization process. The optimum temperature for embryonic development of *C. gariepinus* was suggested at 28–30ºC (Anpe et al 2017) and another study demonstrated that early development can take place in temperature ranges between 20-35ºC (Haylor & Mollah 1995). A different study found that heat shock can expedite the embryonic development process of *C. gariepinus* (Ali & Othman 2022). Some previously studied features of *C. gariepinus* and their hybrids in the early development stages are presented in Table 2.

Table 1

Different stages of embryonic development in the hybrid cross between *Clarias microstomus* (Ng, 2001) ♂ and *Clarias gariepinus* (Burchell, 1822) ♀ under laboratory conditions

Stage number	*Stage*	*Mean time*	*Description*
1	Mature eggs	0 minutes	The mature eggs freshly extruded were adhesive and olive brown in color.
2	Fertilized eggs	0 minutes	The fertilized eggs expanded within 30 seconds after fertilization as the chorion hardened.
3	Animal and vegetal pole	20 minutes	The yolk expands away from the membrane as the cytoplasm accumulates at the anterior pole to form the animal and vegetal poles.
4	First cleavage	45 minutes	Vertical division of the animal pole to produce two cells
5	Four cell stage	48 minutes	A second division of the cells to produce 4 cells.
6	Thirty two cell stage	1 hour 38 minutes	Cells have divided rapidly with differing sizes to the point they were stacked on top each other.
7	Morula stage	4 hours	Cells continue to divide forming a large number of cells grouped together at the animal pole.
8	Blastula stage	5 hours	Cells cover the outline of the yolk.
9	Gastrula stage	9 hours	Cephalic and caudal edges can be seen with rings forming on the developing embryo.
10	26 somite cells	18 hours 5 minutes	Developing embryo starts to move.
11	Hatching	24 hours	The embryo moves it tail vigorously from side to side to rupture the chorion wall and break free.

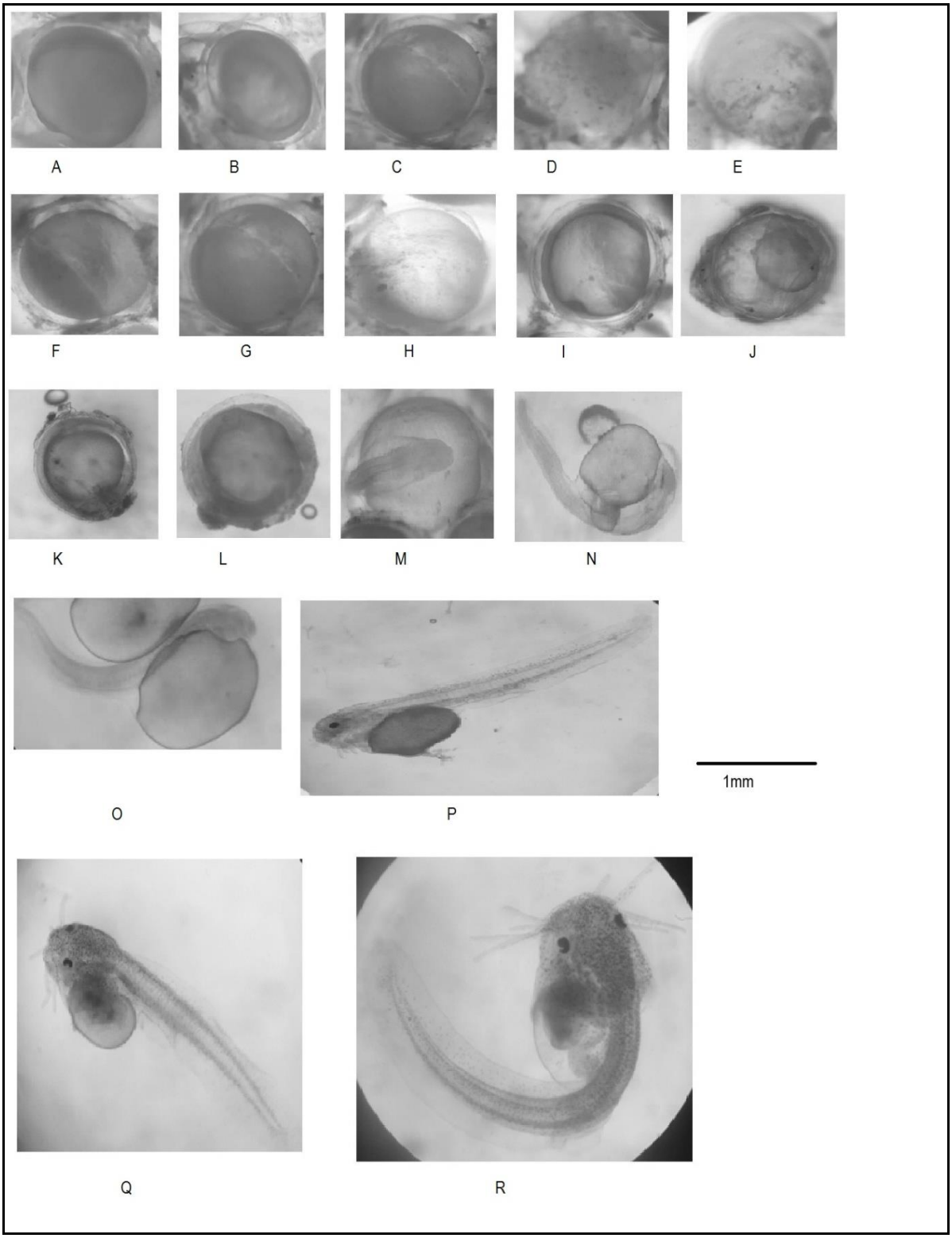

Figure 2. The embryonic development of hybrid offspring between *Clarias microstomus*♂ and *Clarias gariepinus*♀: A - unfertilized egg; B - fertilized egg; C - animal and vegetal poles; D - first cleavage; E - four cell stage; F - thirty two cell stage; G - morula stage; H - blastula stage; I - gastrula stage; J - bud; K - 10 somite cells; L - 21 somite cells; M - 26 somite cells; N - prime; O - newly hatched; P - 24 hour larvae; Q - 48 hour larvae; R - 72 hour larvae.

Table 2

Previous studies and their major findings in different aspects of embryonic development of *Clarias gariepinus* or hybrid varieties

Investigation	*Country*	*Findings*	*Reference*
Embryonic development of *C. gariepinus*	Malaysia	Abnormal cleavages	Hassan et al (2018)
Embryonic development of *C. gariepinus* in laboratory	Nigeria	General observation	Sule & Adikwu (2004)
Embryonic development of *C. gariepinus* under various temperatures	Russia	Incubation at below optimum temperature might increase survivability	Alexandrova et al (2021)
Embryonic development of *C. gariepinus* under various temperatures	Nigeria	Water temperature of 28-30ºC was recommended	Anpe et al (2017)
Embryonic development of crosses between *Pangasianodon hypophthalmus* and *C. gariepinus*	Malaysia	Unequal cell cleavages leading to different forms of deformity	Okomoda et al (2017a)
Early stage and larval development of *C. gariepinus*	Nigeria	First significant chronological developmental stages of *C. gariepinus* embryology (author claimed) and 85% hatching rate	Olaniyi & Omitogun (2014b)
Embryonic development of *C. gariepinus*	Algeria	First report from the country	Behmene et al (2022)
Effect of heat shock on the embryonic development of *C. gariepinus*	Malaysia	Heat shock can expedite the embryonic development process	Ali & Othman (2022)
Influence of temperature on early development of *C. gariepinus*	United Kingdom	Early development can take place when temperature ranges between 20-35ºC	Haylor & Mollah (1995)
Effect of salinity on embryonic development, hatchability, and growth of *C. gariepinus*	Nigeria	Deformity in gastrula stage ranged between 10.4-71.6% treated with 0-10 PSU salinity	Borode et al (2002)
Embryonic development of *Clarias microstomus*♂ and *Clarias gariepinus*♀ hybrid	Malaysia	No deformities found in any stages	Present study

Conclusions. The relative ease in hybridization between *C. microstomus*♂ and *C. gariepinus*♀ may be the result of the close the genetic relationship between both these species. The embryonic development of the hybrid offspring in the present study does not significantly differ from prior reports of pure strains and hybrids of other clariids in terms of both chronology and stages. Thus, the study provides evidence of the potential for developing local hybrid catfish with potentially higher market value for use in aquaculture.

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Conflict of Interest. The authors declare that there is no conflicr of interest.

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