1   **Parasitic cuckoo catfish exploit parental responses to stray offspring**

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3   M. Polačik[1], M. Reichard[1], C. Smith[1,2,3], R. Blažek[1]

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5   [1] The Czech Academy of Sciences, Institute of Vertebrate Biology, Květná 8, 603 65 Brno,

6   Czech Republic

7   [2] Department of Ecology and Vertebrate Zoology, University of Łódź, Łódź, Poland

8   [3] School of Biology and Bell-Pettigrew Museum of Natural History, University of St

9   Andrews, St Andrews, Fife KY16 9TS, UK

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12   **Summary**

13   Interspecific brood parasitism occurs in several independent lineages of birds and social insects,

14   putatively evolving from intraspecific brood parasitism. The cuckoo catfish, *Synodontis*

15   *multipunctatus*, the only known obligatory non-avian brood parasite, exploits mouthbrooding

16   cichlid fishes in Lake Tanganyika, despite the absence of parental care in its evolutionary

17   lineage (family Mochokidae). Cuckoo catfish participate in host spawning events, with their

18   eggs subsequently collected and brooded by parental cichlids, though they can later be

19   selectively rejected by the host. One scenario for the origin of brood parasitism in cuckoo catfish

20   is through predation of cichlid eggs during spawning, eventually resulting in a spatial and

21   temporal match in oviposition by host and parasite. Here we demonstrate experimentally that,

22   uniquely among all known brood parasites, cuckoo catfish have the capacity to re-infect their

23   hosts at a late developmental stage following egg rejection. We show that cuckoo catfish

24   offspring can survive outside the host buccal cavity and re-infect parental hosts at a later

25   incubation phase by exploiting the strong parental instinct of hosts to collect stray offspring.

26   This finding implies an alternative evolutionary origin for cuckoo catfish brood parasitism, with

27   the parental response of host cichlids facilitating its evolution.

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Introduction

Brood parasitism provides some of the best examples of coevolutionary arms races in nature. Brood parasites avoid costs associated with reproduction by exploiting the parental care of their hosts, whereas hosts are selected to avoid the loss of fitness imposed by brood parasites through evolving defences against exploitation. The study of avian brood parasite systems in particular has illustrated a number of mechanisms by which host defences and subsequent parasite counteradaptations can evolve [1,2]. For example, hosts recognize parasitic eggs and chicks on the basis of visual [3,4] and olfactory [5] cues and can reject parasitic eggs [6,7] and chicks [8,9]. In turn, avian brood parasites show the evolution of sophisticated behavioural repertoires [10], morphological adaptations [11], and egg and chick mimesis [12,13] to overcome host defences.

The wealth of information available on avian brood parasitism [1,2,10,14,15] is in sharp contrast with the scarcity of data on the only recognized obligatory non-avian vertebrate brood parasite, the cuckoo catfish *Synodontis multipunctatus* Boulenger 1898. The cuckoo catfish is endemic to African Lake Tanganyika where it coexists with many species of mouthbrooding cichlid fishes [16]. Mouthbrooding is an advanced mode of parental care in fishes in which the eggs are incubated in the buccal cavity of a parent and where hatched offspring are subsequently protected. The spawning rituals of mouthbrooding cichlids involve elaborate courtship and repeated release of small batches of eggs that are quickly collected in the buccal cavity of one or both parents (typically the female) [17]. In Lake Tanganyika, spawning by cichlids can be interrupted by groups of cuckoo catfish, which join the spawning pair of cichlids and deposit their own eggs [16]. In the subsequent mêlée, the parental cichlid frequently collects the eggs of the cuckoo catfish together with its own (e.g. Movie S2 in [18]). Catfish eggs are nonmimetic and typically smaller and rounder than the eggs of Tanganyikan mouthbrooders [19]. Cichlid and catfish eggs are subsequently incubated together in the buccal cavity of the parental cichlid

58   where they are protected from predators. Cichlid eggs hatch within one week but remain in the

59   buccal cavity for an additional 1-2 weeks until they deplete their yolk sacs and start exogenous

60   feeding [20]. The hatching of catfish eggs precedes that of the host cichlid. Once young catfish

61   deplete their yolk, at about 6 days post fertilisation [18], they start feeding on the host embryos.

62   By preying on the young cichlids, the catfish compromises the reproductive success of the host,

63   often consuming the host clutch entirely. Thus, a final outcome of incubation may be a mixed

64   brood comprising both cuckoo catfish and cichlids, but more typically just catfish [18].

65   While the contribution of avian systems to our understanding of brood parasitism is

66   substantial, the opportunity to research a system with a different evolutionary origin may

67   provide a broader understanding of how selection shapes host-parasite coevolution [14]. The

68   catfish-cichlid system is also much more amenable to laboratory research, enabling substantial

69   experimental manipulation [18,21,22]. Like many avian brood parasites, cuckoo catfish

70   eliminate host progeny, though in the case of the cuckoo catfish this is achieved through direct

71   predation [22]. Indeed, the cichlid host provides the parasite with both food and protection while

72   incubation itself appears less critical compared to egg incubation in birds. Because of the

73   necessity of simultaneous spawning with the host, cryptic infestation [14] is impossible and

74   adult cuckoo catfish are always exposed to potential aggression from the host. Unlike in birds,

75   however, where obligatory brood parasitism likely evolved from intra-specific brood parasitism

76   [14,23], the cuckoo catfish (and its related species) perform no parental care, implying that the

77   origin of brood parasitism in the cuckoo catfish may differ markedly from that in birds.

78   In a recent laboratory study, we demonstrated that females of a sympatric host cichlid

79   *Simochromis diagramma* (Günther 1894) can selectively eliminate cuckoo catfish eggs by

80   ejecting them from their buccal cavity while retaining their own brood, with rejection rates of

81   parasite eggs extremely high (90%) [18]. In contrast to avian egg incubation, in which

82   temperature is a limiting factor for survival of eggs and nestlings outside the nest, the aquatic

83 environments inside and outside the mouth of a host cichlid are similar and mouthbrooding

84 primarily protects offspring from predation [20]. In many mouthbrooding cichlids, parents

85 frequently release their offspring from their buccal cavity to forage and collect them back into

86 their mouth upon sighting a predator [17]. Consequently, we hypothesized that rejected cuckoo

87 catfish eggs may have the capacity to survive and hatch in the external environment and

88 subsequently infect their host when collected as a stray offspring by a brooding parent. We

89 conducted three experiments that tested: (1) the ability of cuckoo catfish to develop outside the

90 host buccal cavity, (2) whether hatched cuckoo catfish offspring actively seek a host after

91 rejection, and (3) the propensity of host females to accept the early and late incubation stages

92 of cuckoo catfish from the environment.

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94 **Material and Methods**

95 Experimental fish

96 Four fish species were used in experiments and were maintained under identical

97 conditions (water temperature 26-28 °C, water conductivity 550 µS cm^{-1}, 13:11 light:dark

98 photoperiod). Cuckoo catfish eggs and early juveniles originated from 10 pairs of adults

99 imported from Lake Tanganyika in 2012 and 40 pairs of their F1 progeny. We used *in vitro*

100 fertilisation (see [18]) to produce catfish eggs. The fertilised eggs were either directly used in

101 the experiment or incubated in plastic incubators (tumblers representing an artificial buccal

102 cavity and made of 120 x 15 mm tubing with an inflow rate of 0.25 l min^{-1}) to obtain

103 experimental juveniles (see below). Juveniles were fed live *Artemia* sp. nauplii once each day.

104 A sympatric natural host of cuckoo catfish [16], the mouthbrooding Lake Tanganyika

105 cichlid *Simochromis diagramma*, were obtained from a commercial seller. All adult fish (N =

106 72) were individually marked with Passive Integrated Transponder tags

107 (www.oregonrfid.com), housed in three 350 l mixed sex tanks (4M:20F) and fed with dry and

108 frozen commercial fish food. Aquaria were checked daily for the presence of recently mated

109 (<24 h) females; readily identified by their extended buccal cavity. Brooding females were

110 gently transferred into a 54 l treatment aquarium equipped with an air-driven sponge filter and

111 a 150 mm ceramic cave as a refuge. There the female either underwent an experimental

112 treatment (see below) or served as a source of experimental embryos for control replicates.

113 The Lake George mouthbrooding cichlid *Haplochromis aeneocolor* Greenwood 1973

114 was used as an experimental allopatric host and were obtained from a commercial seller. The

115 allopatric host was used as a control to isolate evolved host responses resulting from the

116 coevolution between the cuckoo catfish and its sympatric hosts. They were housed in three 350

117 l aquaria at a sex ratio of 6M:20F (N = 78) and were otherwise treated in the same way as *S.*

118 *diagramma* in terms of individual tagging, feeding, brooding female checks and subsequent

119 experimental procedures.

120 Allopatric South American Sterba's corydoras *Corydoras sterbai* Knaack 1962 were

121 obtained from a commercial seller and were used as a taxonomically and geographically

122 unrelated control to the juvenile cuckoo catfish. Parental fish were housed in 140 l aquarium

123 where they spawned naturally. The eggs were removed from the aquarium and briefly raised on

124 an *Artemia* nauplii diet until their use in the experiment (see below).

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126 Experiment 1: Host and parasite egg survival outside the female buccal cavity

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128 We experimentally tested the survival of cichlid and cuckoo catfish eggs outside their

129 normal incubation environment; i.e. the buccal cavity of a parental cichlid. Based on our

130 previous finding on the ability of the sympatric cichlid host *S. diagramma* to reject catfish eggs

131 [18] we predicted that selection could favour cuckoo catfish to hatch and commence feeding

132 after rejection by a host. In contrast, we predicted high pre-hatching mortality of the cichlid

133 eggs.

134   A total of 1,448 cuckoo catfish eggs were obtained through *in vitro* fertilisations (IVF) (see

135 [18] for details on the IVF). Each IVF event involved multiple parental fish (2-5 females, 3-5

136 males) to produce genetically variable offspring. Fertilised eggs were split into two groups. The

137 treatment group eggs (599 eggs) were transferred to 64 L aquaria equipped with a 400 l h^{-1}

138 power filter and 0.75 l min^{-1} aeration. Each egg was placed into a single cell (20 by 20 mm, 20

139 mm deep) of a 5 x 5 compartmentalised plastic dish on a 5 mm layer of fine sand in each

140 compartment and observed daily. A total of 24 independent replicates (clutches) were

141 completed, using 25 eggs per each replicate (with a single exception of 24 eggs in one replicate).

142 In the first 10 replicates (250 eggs in total), we recorded survival to the age of 72 h (to

143 standardise comparison with host development), time to hatching (duration of pre-hatching

144 development) and survival to hatching (hatching success). The same data were recorded in an

145 additional 14 clutches (349 eggs in total) but with a follow-up observation on the first day of

146 external feeding to measure the proportion of juveniles that started to feed successfully. Control

147 cuckoo catfish eggs originated from the same IVFs as the first 10 replicates, with 27-191 eggs

148 per replicate (849 control eggs in total). Control eggs were placed in artificial incubators that

149 ensured constant movement of the eggs to imitate conditions in the buccal cavity of a host. The

150 eggs that were found not to be developing during the first inspection; i.e. 24 h after fertilisation,

151 were regarded as unfertilised. Mean fertilisation rate did not differ between incubators and

152 aquaria (p = 0.89) and was 45.6% and 44.0% in the incubators and aquaria, respectively.

153   Cichlid eggs were incubated using the same protocol as for catfish eggs. A total of 317 *S.*

154 *diagramma* eggs (14 replicates) and 595 *H. aeneocolor* eggs (24 replicates) that originated from

155 natural spawning (see Experimental fish) were tested. Brooding females of each species that

156 had spawned within the previous 6 h were gently stripped of their fertilised eggs [18]. As for

157 the cuckoo catfish eggs, survival until hatching was scored from the eggs that were alive after

158 24 h, accounting for unfertilised eggs. The rate of fertilisation (after 24h) was 79.4% in *S.*

159 *diagramma* and 73.3% in *H. aeneocolor*.

160   The survival of cuckoo catfish and host embryos outside the buccal cavity until hatching

161 were expressed as a bivariate vector (ratio of surviving to fertilised eggs for each clutch) and

162 differences between cuckoo catfish and sympatric and allopatric cichlids were tested using a

163 Generalized Linear Model with binomial error distribution and log-link function in the *glm*

164 package in the R statistical environment [24]. Given that hatching in cichlids occurred later than

165 hatching in the cuckoo catfish (3 vs. 6 days), as an additional control we tested survival over

166 the first 3 days of incubation to accommodate this disparity in time to hatching. We also

167 compared the hatching success of cuckoo catfish eggs between a sand substrate (treatment) and

168 incubator (control) using a Generalized Mixed Model with binomial error in the *lme4* package.

169 This analysis included clutch ID as a random term to account for a paired design in the data,

170 since clutches were split between the two incubation methods. Duration of pre-hatching

171 development was tested on the same dataset, using the same GLMM procedure but with a

172 Poisson error distribution (number of days).

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174 Experiment 2: Behaviour of parasite offspring in the presence of a brooding host female

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176   We tested the behavioural response of free-swimming cuckoo catfish offspring to the

177 presence of a brooding cichlid female. We predicted that juvenile catfish would actively seek

178 brooding host females to increase the probability of being collected and brooded, manifested as

179 a positive spatial association between brooding female and the free-swimming parasite juvenile.

180   A 120 l aquarium (750 x 400 x 400 mm) was divided into three equally-sized sections

181 along its longitudinal axis. Both sides of the aquarium were equipped with air driven filters and

182   separated from the central section with transparent plastic dividers. A female *S. diagramma* that

183   had recently spawned (<24 h) was placed in either the left or right lateral section. The dividers

184   restricted the female from entering the central section but enabled full olfactory contact between

185   the test fish through 30 holes (10 mm in diameter) and by positioning the divider 20 mm above

186   the bottom of the tank. A single cuckoo catfish (4-8 days old, median = 6 days, N = 30, mean

187   total length, measured through digital imaging (95% confidence limits) = 13.5 (13.0-14.0 mm))

188   or a control corydoras catfish (10-20 days old, N = 30, mean body size (95% confidence limits)

189   = 13.6 (13.1-14.1 mm)) was placed in the middle of the test aquarium and covered with a

190   transparent pot and allowed to acclimatize. After 5 min, the pot was gently removed and the

191   catfish released. The arrangement of the tank enabled unrestricted movement of the

192   experimental juveniles while time spent in respective sections of the test aquarium was recorded

193   for a period of 45 min. Three individual juvenile cuckoo catfish and three corydoras catfish

194   were tested with each of 10 host females, providing 30 cuckoo catfish replicates and 30 control

195   corydoras replicates.

196   To test whether juvenile cuckoo catfish preferred to associate with the host cichlid, we used

197   a Generalized Linear Mixed Model with Gamma error distribution and identity-link function in

198   the *lme4* package. We tested whether juvenile cuckoo catfish associated with the brooding host

199   female more often than control corydoras juveniles, and whether the cuckoo catfish spent more

200   time in the preference compartment than would be expected at random (i.e. 33% of time). The

201   analysis included female ID as a random term to account for repeated use of the same females

202   over six successive replicates.

203

204   Experiment 3: parasite re-infection of the host

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206   We tested the potential of juvenile cuckoo catfish to re-infect brooding females of their

207   sympatric and allopatric cichlid hosts. We predicted that small, free-swimming cuckoo catfish

208   that were rejected by the host (see [18]) might be able to return to the buccal cavity of a brooding

209   host cichlid by exploiting their strong parental response to recover dropped or stray offspring.

210   Naturally spawned brooding females of both sympatric *S. diagramma* and allopatric *H.*

211   *aeneocolor* were transferred to treatment aquaria (see Experimental fish). These fish were

212   presented with cuckoo catfish and conspecific offspring for a period of 48 h. After exposure,

213   all offspring were gently washed out of the buccal cavity of the host to determine whether the

214   female had accepted the experimentally exposed offspring or consumed them. In order to

215   disentangle the effect of host brooding stage on host response, experimental exposure took place

216   before hatching (i.e. at the egg incubation phase, with trials starting 0-1 day post fertilisation in

217   both cichlid species) or after hatching (embryo incubation phase, *S. diagramma*: starting 14-15

218   days post fertilisation; *H. aeneocolor*: starting 8-9 days post fertilisation given its more rapid

219   development).

220   At the host egg incubation phase, a total of 20 *S. diagramma* and 20 *H. aeneocolor* brooding

221   females were used. Each female was used only once. We presented 10 females of each host

222   species with five juvenile cuckoo catfish (age 1-6 days post hatching) and an additional 10

223   females with 4-6 non-swimming embryos of their own species, obtained from a non-

224   experimental female (age 2-8 and 2-7 days post hatching in *S. diagramma* and *H. aeneocolor*,

225   respectively). Experimental aquaria were visually isolated from external cues for a period of 48

226   h. During trials juvenile catfish were provided with 2 ml of live *Artemia* nauplii suspension

227   once each day.

228   At the embryo incubation phase, the same protocol was used but high acceptance rates of

229   conspecific and parasitic offspring (see Results) prompted inclusion of an additional,

230   geographically and taxonomically unrelated control group. Thus, an additional 10 S.

231   *diagramma* and 10 *H. aeneocolor* females were presented with five juvenile corydoras (aged

232   10-20 days post hatching to match the cuckoo catfish body size) following an identical protocol

233   to that for cuckoo catfish.

234   Each host female was only used once at a particular incubation phase, though there was

235   partial overlap (65%) of females used between incubation phases. To distinguish conspecific

236   experimental offspring from the test female's own offspring at the embryo incubation phase,

237   experimental offspring were lightly stained using a 1 hour bath in Alizarin Red solution, freshly

238   prepared before each replicate by diluting 150 mg of Alizarin Red dye in 1 litre of tank water

239   at 26°C.

240   After 48 h each experimental host cichlid female was gently netted out of the aquaria and

241   the entire contents of her buccal cavity washed out [18]. The number of juveniles and embryos

242   inside the buccal cavity was recorded. The offspring that remained in the aquarium (i.e. those

243   not accepted by the experimental female) were also netted and counted. For conspecific

244   treatments, all embryos were inspected using a binocular microscope under fluorescent light

245   (wave length 532 nm). Alizarin-stained individuals were identified from their fluorescently red

246   skeletal structures [25].

247   To compare re-infection rates between sympatric and allopatric host species and among

248   young stages of cuckoo catfish, conspecific control and catfish control (corydoras), we used a

249   Generalized Linear Model with binomial error distribution and log-link function. Given the

250   repeated use of a subset of females for the egg and embryo incubation phases (but while

251   incubating different clutches), we analysed the two datasets separately. Re-infection rates were

252   calculated as a bivariate vector (ratio of accepted offspring to offered offspring); the number of

253   offered offspring was typically 5 but varied between 4 (5 replicates) and 6 (1 replicate). A quasi-

254   binomial error structure was used for data from the egg incubation phase given a high incidence

255   of zero acceptance rates. Saturated models included host species (sympatric, allopatric) and

256   offspring species (cuckoo catfish, conspecific and corydoras in the embryo incubation phase

257   dataset) and their interactions. Interactions between host and offspring were always non-

258   significant and were removed from the final models. For each treatment group, the proportion

259   of embryos accepted by a female and the proportion of host females that collected at least a

260   single embryo (acceptor hosts) were calculated.

261

262   **Results**

263   Experiment 1: Host and parasite survival outside the female buccal cavity

264   The success of incubation on a sand substrate was good for cuckoo catfish but poor in

265   cichlids (Figure 1). Hatching success in cuckoo catfish was 78%, but only 1.5% in both

266   sympatric and allopatric hosts (GLM with binomial distribution: $\chi^2 = 33.1$, df $= 2$, p < 0.001, N

267     $= 61$ clutches). This difference remained after controlling for an unequal embryo developmental

268   time in cuckoo catfish and cichlids; on day 3 post fertilisation on a sand substrate catfish egg

269   survival was 78.5% but only 15% for allopatric and 3.5% for sympatric host eggs ($\chi^2 = 87.7$,

270   df $= 2$, p < 0.001). All hatched cuckoo catfish started to feed exogenously (at day 7 post

271   fertilisation, N $= 152$ fish from 14 clutches), as did all cichlids (the day of first feeding not

272   recorded).

273   There was no difference in cuckoo catfish egg survival to hatching on sand in comparison

274   with eggs raised in an artificial incubator (GLMM with binomial error: z $= 0.60$, p $= 0.269$, n $=$

275   10 paired samples) and no difference in the time to hatching (GLMM with a Poisson error: z $=$

276   0.14, p $= 0.89$, n $= 10$). Catfish eggs typically hatched in 3-4 days (day 3: 16 clutches, day 4:

277   16 clutches), with a single clutch hatching on day 2. All eggs from the same clutch always

278   hatched synchronously on the same day.

279

280   Experiment 2: Parasite juvenile behaviour in the presence of a brooding host female

281   Juvenile cuckoo catfish were not attracted by brooding sympatric host females. There

282   was no difference in association with brooding host females between the cuckoo catfish and

283   corydoras juveniles (GLMM with Gamma distribution, $z = 0.60$, $p = 0.547$, $N = 30$ juveniles

284   per treatment). Time spent by cuckoo catfish juveniles in each compartment was similar, while

285   corydoras showed a tendency to avoid the central compartment (Fig. 2).

286

287   Experiment 3: Parasite re-infection of the host

288   Both cichlid species accepted hatched heterospecific and conspecific offspring, though

289   sympatric females did so at a higher rate. Host females showed a greater propensity to collect

290   offspring at the embryo brooding stage than at the earlier egg brooding stage (Fig. 3).

291   While incubating their own unhatched eggs, acceptance rates of heterospecific and

292   conspecific offspring were low, with no difference between sympatric and allopatric cichlids

293   (GLM with quasibinomial error, $z = 0.01$, $p = 0.994$), nor between unrelated conspecific and

294   parasite offspring ($z = 1.30$, $p = 0.194$). Sympatric females accepted 22% of cuckoo catfish

295   juveniles (3 out of 10 females accepted at least a single juvenile; i.e. were acceptors) and 10%

296   of conspecific embryos (a single acceptor out of 10). No allopatric females accepted any

297   conspecific or parasitic offspring at the egg incubation stage (Fig. 3a).

298   When incubating their own hatched embryos, the acceptance rate was significantly higher in

299   sympatric females (GLM with binomial error, $z = 3.14$, $p = 0.002$) and differed among offspring

300   species ($z = 5.49$, $p < 0.001$). Acceptance was low in corydoras catfish (sympatric hosts: 14%

301   offspring collected, 40% acceptor females; allopatric hosts: 6% offspring, 20% acceptors), but

302   high in parasitic cuckoo catfish (sympatric hosts: 94% offspring collected, 100% acceptor

303   females; allopatric hosts: 38% offspring, 50% acceptors) and conspecific embryos (sympatric

304   hosts: 84% offspring collected, 100% acceptor females; allopatric hosts: 58% offspring, 100%

305   acceptors) (Fig. 3b).

306

Discussion

We showed that the eggs and embryos of parasitic cuckoo catfish are capable of surviving at high rates outside the buccal cavities of their hosts, at least in a laboratory setting. Further, a strong parental response by both allopatric, but especially sympatric, hosts (Fig. 3), provided actively swimming cuckoo catfish offspring (1-6 days post hatching) with an opportunity to parasitize hosts long after oviposition. Parental females of both tested cichlid species readily collected cuckoo catfish offspring, as well as control conspecific embryos and, to a lesser extent, offspring of geographically distinct corydoras catfish when incubating their own hatched embryos. Acceptance rate was much lower during the egg incubation phase. In contrast to our predictions, we detected no directional behavioural response by cuckoo catfish offspring to brooding host females, suggesting that they do not actively seek potential hosts.

The implications of our study are that, uniquely among all known brood parasites, cuckoo catfish have the capacity to infect hosts at two qualitatively different ontogenetic stages; as an egg and later as an actively swimming juvenile. Hence, even after rejection at the egg stage, juvenile cuckoo catfish could complete development to the free-swimming stage and return to the buccal cavity of a host, at least under the conditions imposed in this study. Indeed, the ability to reject parasitic eggs, but not to discriminate against juvenile parasites (but see [26,27]) resembles the situation seen in many avian brood parasite systems. This situation can be explained under a number of alternative hypotheses (reviewed by [28]), but probably arises through low selection pressure imposed by a low frequency of occurrence of parasite offspring following frequent rejection [28,29].

The ability of juvenile cuckoo catfish to re-infect hosts appears to derive primarily from a parental response of the hosts to collect stray offspring, rather than from juvenile cuckoo catfish actively seeking to re-infect the host. The presence of non-swimming embryos and, notably,

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331 unrelated non-parasitic corydoras in the mouth of brooding females strongly suggests that re-

332 infection is accomplished by the host actively collecting free-swimming juveniles. Our data

333 also show that the motivation to collect the fish is higher when the offspring in the buccal cavity

334 have already hatched. This finding suggests that mouthbrooding females can reference the

335 developmental status of their broods and modify their behaviour in response. The cost of

336 parasitism at this later stage can be either lower or higher than when the brood is infected at the

337 time of spawning and depends on the timing of acceptance and the number and size of the

338 accepted parasitic offspring. In many cases, parental host cichlids may be unable to distinguish

339 their own offspring from unrelated or even heterospecific young. Mobile young stages often

340 stray from their parents, or are displaced when predators attack a parent or brood. The

341 inadvertent adoption of such young by unrelated parents is probably not uncommon in teleost

342 fishes [20,21]. The costs of policing care by parents, including expelling unrelated offspring, is

343 potentially expensive if the error rate in discriminating genetically related and unrelated young

344 is significant. In addition, if the fitness cost to a parent of caring for small numbers of genetically

345 unrelated offspring is trivial, the strength of selection to evolve mechanisms to discriminate and

346 expel unrelated young may be limited. Parasite infection facilitated by the host itself is also

347 known in the butterfly *Phengaris arion* whose larvae parasitise ant colonies. However, *P. arion*

348 larvae manipulate the ants into carrying them to their nest using chemical and acoustic signals

349 [30] whereas the propensity to accept offspring of other cichlid species is a general feature of

350 many mouthbrooders [21,31,32]. Our tests demonstrated sympatric *S. diagramma* to be a

351 relatively stronger acceptor than the allopatric *H. aeneocolor* but this finding has limited general

352 application as the comparison only included two species. Whether cuckoo catfish similarly use

353 behavioural, visual, olfactory or auditory signals to manipulate hosts into retrieving them is an

354 intriguing possibility that remains to be tested.

355   The active compliance by hosts in their own infection by cuckoo catfish as a by-product of

356   parental care also offers a hypothetical trajectory for the origin of this brood–parasitic

357   relationship. While the evolution of obligatory brood parasites in birds is believed to originate

358   from intra-specific brood parasitism (e.g. [23,33]), this scenario is not plausible in *Synodontis*

359   catfishes since they belong to a lineage that lacks parental care. One scenario for the evolution

360   of brood parasitism in cuckoo catfish could be through predation of cichlid eggs during

361   spawning, which might eventually result in a spatial and temporal match of spawning by both

362   the parasite and its host. The results of the present study, however, suggest an alternative

363   evolutionary pathway, with the relationship potentially evolving through accidental incubation

364   of ancestral cuckoo catfish juveniles by brooding cichlids, with the fitness benefits of

365   mouthbrooding reinforcing a spatial and temporal association of the catfish with cichlid hosts.

366   This hypothesised evolutionary pathway is analogous to the widely accepted theory for the

367   evolution of trophically transmitted parasites from free-living species (e.g. [34]).

368   Cuckoo catfish eggs and juveniles showed high survival rates outside the buccal cavity of

369   the host, potentially weakening reliance by the parasite on the host, especially in comparison

370   with the negligible survival of cichlid embryos. Simultaneous spawning by cuckoo catfish and

371   cichlids involves aggressive behaviour by the spawning cichlid pair with the catfish often forced

372   away from the spawning site [21]. Even in the confines of an aquarium setting, catfish and

373   cichlid eggs can be swept away from the spawning arena during aggressive disputes (M.

374   Polačik, R. Blažek pers. obs.). Under these conditions, some uncollected cuckoo catfish eggs,

375   as well as the eggs rejected by a host female, may be able to survive, hatch and develop along

376   an alternative, non-parasitic developmental pathway. Cuckoo catfish are considered an obligate

377   brood parasite in the scientific literature (e.g. [16,18, 21,22]), though evidence from the wild is

378   indeterminate being based solely on the failure, thus far, to detect juvenile cuckoo catfish

379   outside the care of their hosts [16]. Our own observations from captivity (M. Polačik, R. Blažek

pers. obs.) and anecdotal information from fish hobbyists suggest that cuckoo catfish can

occasionally reproduce without parasitizing cichlids, though whether outcomes in the benign

environment of the aquarium necessarily translate to nature is clearly a question that needs to

be addressed. Obligate brood parasitism is believed to typically evolve along a trajectory

starting with facultative parasitism (e.g. [23]) and it is conceivable that cuckoo catfish have yet

to complete the transition to the full, obligate brood parasitism. There is also a possibility that

different populations of the cuckoo catfish, which is widespread across Lake Tanganyika [35]

may express different levels of reliance on their hosts.

In conclusion, the relationship between the cuckoo catfish and mouthbrooding cichlids

represents a unique example of a versatile vertebrate brood-parasitic system that is unusually

amenable to experimental manipulation. We present data suggesting that cuckoo catfish

offspring can complete development without exploiting a host, at least in a laboratory setting

when predation is excluded. In addition, a strong parental response by mouthbrooding cichlids

to collect stray offspring may facilitate re-infection of hosts by cuckoo catfish juveniles after

rejection at the egg stage, and may even represent an evolutionary pathway for brood parasitism

by cuckoo catfish with the strong parental instinct of host cichlids facilitating the origin of brood

parasitism.

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Data Accessibility

Primary data will be deposited on the Figshare repository.

Authors' Contributions

The study was conceived by M.P. and R.B. and designed by M.P., R.B. and M.R. Data collection: M.P. and R.B. Data analysis: M.R. Data interpretation: M.P., C.S., R.B. and M.R. Drafting ms: M.P. with important contributions made by all authors.

**Competing Interests**

None.

Fig. 1. Hatching success of parasite and host eggs when incubated outside female buccal cavity. Proportion of cuckoo catfish and host eggs that successfully hatched on a sand substrate and at artificial buccal cavity (incubator). Median, interquartile range and non-outlier range are shown, along with replicate-specific values clutches of 25 eggs (black circles). The number of replicates (clutches) is shown for each treatment.
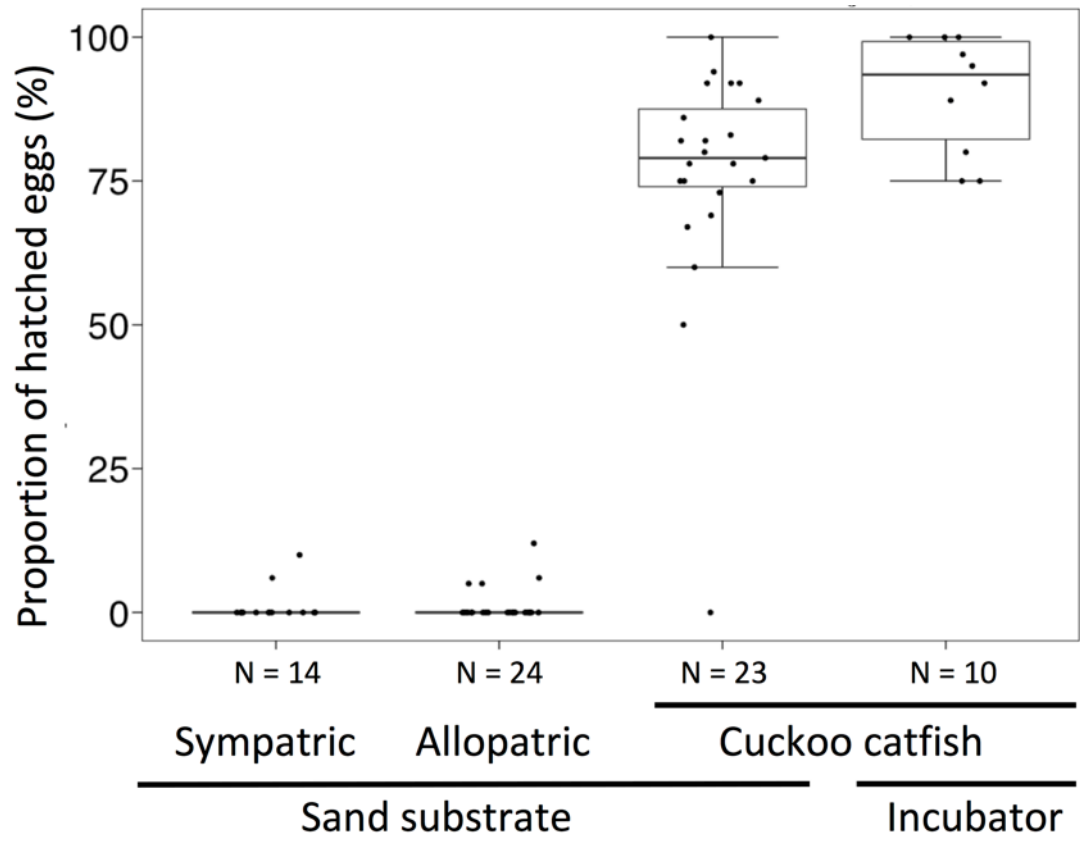
Fig. 2. Catfish behaviour towards brooding host female. Median time spent in each compartment (preference, neutral and avoidance zones), with interquartile range (box) and non-outlier range (whiskers). Individual values are shown as black circles.
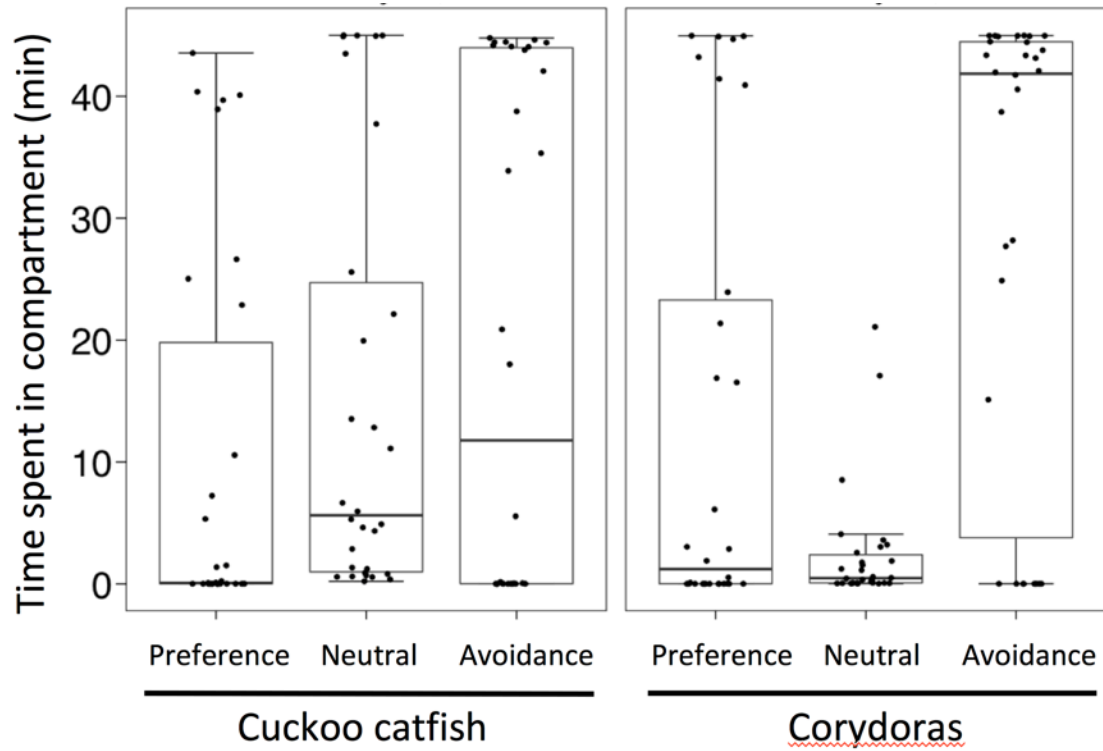
Fig. 3. Acceptance of embryos by host females at egg (left panels) and embryo (right panels) incubation phases. Median, interquartile range and non-outlier range are shown for each tested combination of host and parasite species, with replicate-specific values for each trial (consisting of 4-6 embryos) (black circles).