

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

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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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29 **Keywords:** brood parasite, Cichlidae, coevolutionary arms race, host-parasite evolution

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33 **Introduction**

34 Brood parasitism provides some of the best examples of coevolutionary arms races in nature.
35 Brood parasites avoid costs associated with reproduction by exploiting the parental care of their
36 hosts, whereas hosts are selected to avoid the loss of fitness imposed by brood parasites through
37 evolving defences against exploitation. The study of avian brood parasite systems in particular
38 has illustrated a number of mechanisms by which host defences and subsequent parasite
39 counteradaptations can evolve [1,2]. For example, hosts recognize parasitic eggs and chicks on
40 the basis of visual [3,4] and olfactory [5] cues and can reject parasitic eggs [6,7] and chicks
41 [8,9]. In turn, avian brood parasites show the evolution of sophisticated behavioural repertoires
42 [10], morphological adaptations [11], and egg and chick mimesis [12,13] to overcome host
43 defences.

44 The wealth of information available on avian brood parasitism [1,2,10,14,15] is in sharp
45 contrast with the scarcity of data on the only recognized obligatory non-avian vertebrate brood
46 parasite, the cuckoo catfish *Synodontis multipunctatus* Boulenger 1898. The cuckoo catfish is
47 endemic to African Lake Tanganyika where it coexists with many species of mouthbrooding
48 cichlid fishes [16]. Mouthbrooding is an advanced mode of parental care in fishes in which the
49 eggs are incubated in the buccal cavity of a parent and where hatched offspring are subsequently
50 protected. The spawning rituals of mouthbrooding cichlids involve elaborate courtship and
51 repeated release of small batches of eggs that are quickly collected in the buccal cavity of one
52 or both parents (typically the female) [17]. In Lake Tanganyika, spawning by cichlids can be
53 interrupted by groups of cuckoo catfish, which join the spawning pair of cichlids and deposit
54 their own eggs [16]. In the subsequent mêlée, the parental cichlid frequently collects the eggs
55 of the cuckoo catfish together with its own (e.g. Movie S2 in [18]). Catfish eggs are nonmimetic
56 and typically smaller and rounder than the eggs of Tanganyikan mouthbrooders [19]. Cichlid
57 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.

93

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 $\mu\text{S cm}^{-1}$, 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).

125

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

127

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).

173

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

175

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

205

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 **Results** 262

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

307 **Discussion**

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

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

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

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

380 pers. obs.) and anecdotal information from fish hobbyists suggest that cuckoo catfish can
381 occasionally reproduce without parasitizing cichlids, though whether outcomes in the benign
382 environment of the aquarium necessarily translate to nature is clearly a question that needs to
383 be addressed. Obligate brood parasitism is believed to typically evolve along a trajectory
384 starting with facultative parasitism (e.g. [23]) and it is conceivable that cuckoo catfish have yet
385 to complete the transition to the full, obligate brood parasitism. There is also a possibility that
386 different populations of the cuckoo catfish, which is widespread across Lake Tanganyika [35]
387 may express different levels of reliance on their hosts.

388 In conclusion, the relationship between the cuckoo catfish and mouthbrooding cichlids
389 represents a unique example of a versatile vertebrate brood-parasitic system that is unusually
390 amenable to experimental manipulation. We present data suggesting that cuckoo catfish
391 offspring can complete development without exploiting a host, at least in a laboratory setting
392 when predation is excluded. In addition, a strong parental response by mouthbrooding cichlids
393 to collect stray offspring may facilitate re-infection of hosts by cuckoo catfish juveniles after
394 rejection at the egg stage, and may even represent an evolutionary pathway for brood parasitism
395 by cuckoo catfish with the strong parental instinct of host cichlids facilitating the origin of brood
396 parasitism.

397

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488

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492 **Ethics**

493 Research adhered to all national and institutional animal care and use guidelines (permit No.
494 CZ62760203)

495 **Data Accessibility**

496 Primary data will be deposited on the Figshare repository.

497 **Authors' Contributions**

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

501 **Competing Interests**

502 None.

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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.

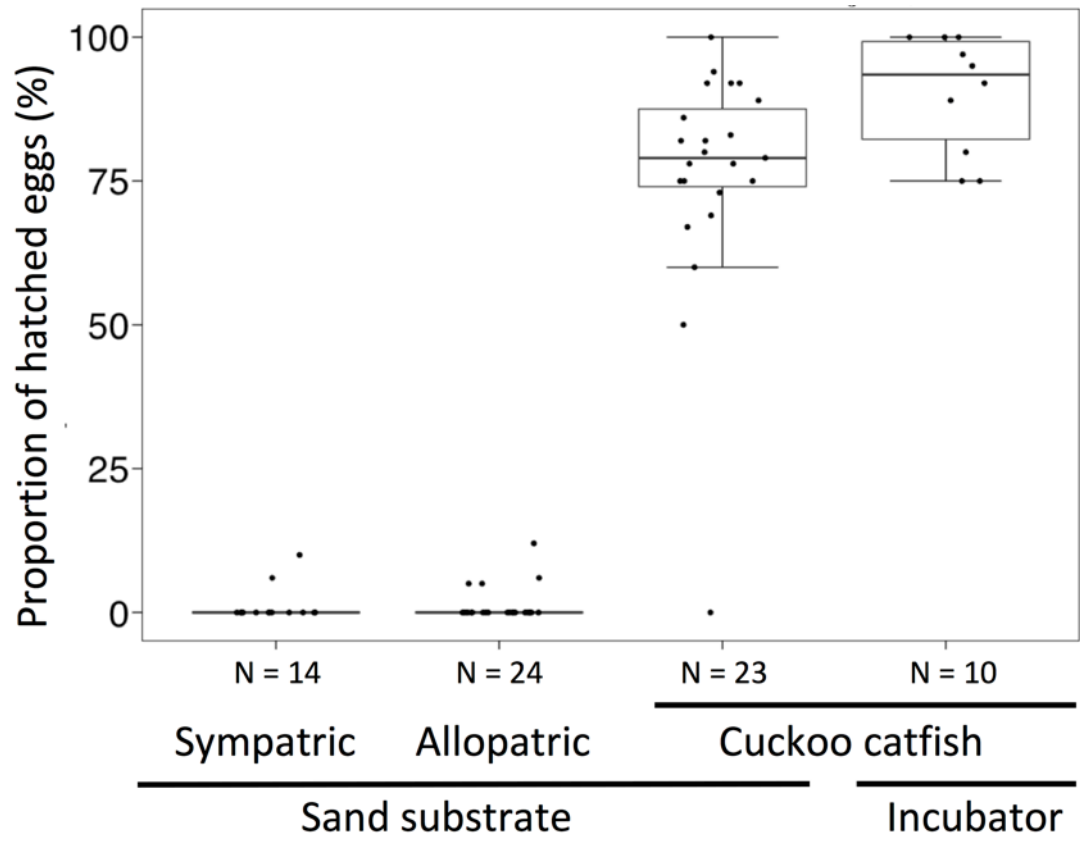


Fig. 2. Catfish behaviour towards brooding host female. Median time spent in each compartment (preference, neutral and avoidance zones), with interquartile range (box) and non-outlier range (whiskers). Individual values are shown as black circles.

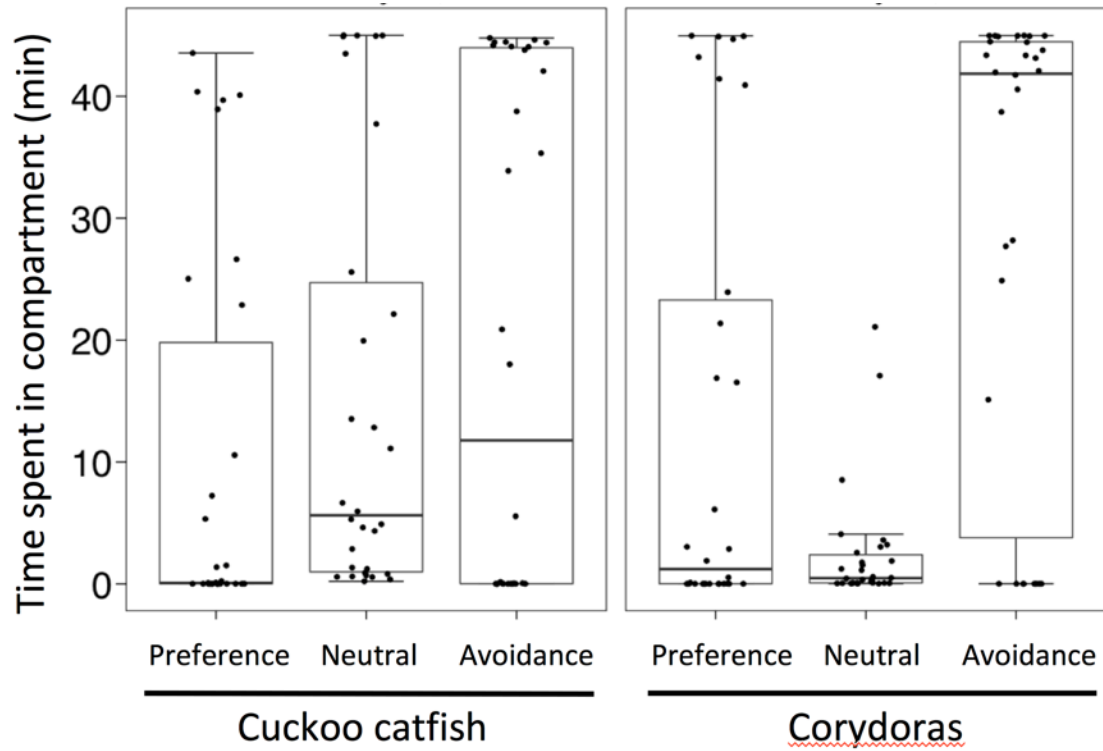


Fig. 3. Acceptance of embryos by host females at egg (left panels) and embryo (right panels) incubation phases. Median, interquartile range and non-outlier range are shown for each tested combination of host and parasite species, with replicate-specific values for each trial (consisting of 4-6 embryos) (black circles).

