1 **Biological Sciences:** Population Biology

2 **Title:** *First genealogy for a wild marine fish population reveals multi-generational*
3 *philopatry*

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23 **Keywords:** *Amphiprion percula* | self-recruitment | multi-generational pedigree | inbreeding |

24 parental effects

25 **Abstract**

26 Natal philopatry — the return of individuals to their natal area for reproduction — has advantages

27 and disadvantages for animal populations. Natal philopatry may generate local genetic adaptation

28 but may also increase the probability of inbreeding that can compromise persistence. While natal

29 philopatry is well documented in anadromous fishes, marine fish may also return to their birth site

30 to spawn. How philopatry shapes wild fish populations is, however, unclear because it requires

31 constructing multi-generational pedigrees that are currently lacking for marine fishes. Here we

32 present the first multi-generational pedigree for a marine fish population by repeatedly genotyping

33 all individuals in a population of the orange clownfish (*Amphiprion percula*) at Kimbe Island

34 (Papua New Guinea) over a 10-year period. Based on 2927 individuals, our pedigree analysis

35 revealed that longitudinal philopatry was recurrent over five generations. Progeny tended to settle

36 close to their parents, with related individuals often sharing the same colony. However, successful

37 inbreeding was rare and genetic diversity remained high, suggesting occasional inbreeding does not

38 impair local population persistence. Local reproductive success was dependent on the habitat larvae

39 settled into, rather than the habitat they came from. Our study suggests that longitudinal philopatry

40 can influence both population replenishment and local adaptation of marine fishes. Resolving multi-

41 generational pedigrees over a relatively short time period, as we present here, provides a framework

42 for assessing the ability of marine populations to persist and adapt to accelerating climate change.

43 **Significance Statement**

44 Evidence for natal philopatry – the return of individuals to their natal location for reproduction – is

45 scarce in marine fish populations despite being common in anadromous fishes. The proportion of

46 individuals returning to natal sites is an important metric for estimating the effects of inbreeding

47 and the potential for local adaptation to generate resilience to climate change. Here, we present the

48 first multi-generational pedigree for a wild marine fish. We resolved the genealogical tree of

49 families of orange clownfish *Amphiprion percula* spanning up to five generations using data from a

50 10-year genetic survey of a population at Kimbe Island, Papua New Guinea. We found that

51 longitudinal philopatry plays a significant role in driving population renewal of the orange

52 clownfish.

53 /body

Introduction

55 The dispersal of immature individuals away from their natal area in search of breeding sites can

56 negatively impact population replenishment and, when not offset by immigration, put small

57 populations at risk of extinction (1). Long-distance dispersal may also reduce the potential for local

58 adaptation to environmental change, as individuals may be poorly adapted to distant habitats and

59 suffer higher rates of mortality prior to reproduction (2). Natal philopatry— the return of

60 individuals to their natal area (3) — provides a mechanism to avoid at least some of the negative

61 consequences of dispersal, because genetically adapted individuals contribute to the renewal of their

62 population of origin. Yet philopatry is a double-edged sword, because although it can contribute to

63 population replenishment, it may also increase inbreeding (3) that often has a deleterious effect on

64 fertility and poses an extinction risk for small populations (4). Inbreeding may also reduce genetic

65 diversity (5) that can reduce the potential of populations to adapt to changing conditions through

66 natural selection (6). Therefore, understanding the balance between dispersal and philopatry is

67 critical in animal populations because of the implications for genetic diversity (7), adaptation (8)

68 and conservation (9).

69 The vast majority of benthic marine species, including most coral reef fishes, have a dispersive

70 larval stage in their early life history before settlement into adult habitat (10). Historically, larval

71 offspring were considered as essentially passive particles carried by ocean currents to locations far

72 from their natal population (11). More recently, it has been demonstrated that, like many

73 anadromous species, marine fish may also return to their birth area (11-15). It has proved more

74 difficult to verify that those individuals returning to their natal location later successfully reproduce

75 as part of the adult population. Philopatric behavior has been widely documented in terrestrial

76   vertebrate species, including birds and large mammals (16), using multi-generational pedigrees of

77   local populations. Multi-generational pedigrees have yet to be constructed for a wild marine fish

78   population, perhaps because of the presumed dissociation between parents, offspring and their

79   relatives (17). However, natal philopatry may be more prevalent in marine species than previously

80   thought. Evidence for self-recruitment in coral reef fishes is accumulating (11-14, 18) and these

81   findings raise questions about the role of philopatry in determining the structure and persistence of

82   local populations. For example, do long-term family lineages contribute to generational turnover in

83   local populations? Are the benefits of natal philopatry offset by the negative effects of inbreeding?

84   Here, we resolve the first multi-family genealogical trees for a wild marine fish population using a

85   10-year genetic survey and evaluate the extent and effects of philopatry on inbreeding.

Results

87   **Pedigree reconstruction.** We found that self-recruitment (*i.e.*, the ratio of locally produced settlers

88   to settlers from all origins arriving at a given settlement site (19)) occurs over multiple generations

89   in a wild population of the orange clownfish, *Amphiprion percula*, on coral reefs around Kimbe

90   Island, Papua New Guinea. Self-recruitment averaged 56% over a 10-year period (20). We followed

91   the fate of every fish (2927 individuals) and their philopatric progeny over 10 years using genotypic

92   profiling and parentage analysis (see methods, Fig. 1*A*). The multi-generational pedigree we

93   reconstructed contained 502 informative founders, 987 parent-progeny links, 1809 full-siblings, 412

94   maternal half-siblings and 248 paternal half-siblings, 278 paternal grand-mothers and grand-fathers,

95   and 135 maternal grand-mothers and grand-fathers (Table 1). The pedigree included up to five

96   generations of individuals within the same family over the 10-year period (Fig. 1*B*, see examples of

97   families in Fig. S1). We found examples of families that spread throughout the Kimbe Island

98   population and others that grouped in small geographic areas around the island (Fig. 2). On the

99   smallest spatial scale, we found 57 instances of family members recruiting to the same anemone

100   (2% of individuals from the local population), including cohabitation of parents and offspring, full-

101   siblings and half-siblings (Table 1). The pedigree reconstruction revealed that 37% of adults (649

102 individuals) contributed nearly 56% to the renewal of the local orange clownfish population every

103 two years (20). Clearly, some individuals contributed more than others to future generations, as is

104 shown in the variety of family tree sizes reconstructed (Fig. S1). Our findings imply that inter-

105 generational continuity in this insular population is dependent on a small proportion of the breeders

106 contributing most of the local replenishment.

107 **Inbreeding and extra-pair mating.** The potential downside of philopatry over multiple

108 generations in marine fish populations is inbreeding; *i.e.* that mating partners are genetically related.

109 Our pedigree reconstruction generally showed that most juveniles from the same family settled in

110 different anemones from parents and siblings. Only two cases of successful inbreeding were

111 observed, one between a brother and sister and one between a grandson and grandmother (Table 1).

112 Whatever the mechanism, inbreeding in the orange clownfish population was rare ($F_{IS} = 0.018$) and

113 average genetic diversity remained high ($H_S = 0.714$, Table S1 for detailed results).

114 Family trees were almost entirely based on adult pairs that co-habit the same anemone. We

115 previously documented the presence of two cases of adults moving from their anemones to another

116 anemone to reproduce (extra-pair mating, (20)). The distance between the anemones of the two

117 parents for these 2 cases were 13 and 133 meters, respectively. Our data indicate extra-pair mating

118 was uncommon, presumably because individual *A. percula* are strongly site-attached and rarely

119 stray more than a few meters beyond the periphery of their anemone (21, 22).

120 **Parental and environmental effects.** We tested the role that parental habitat and environment

121 played in offspring survival and reproduction on Kimbe Island reefs. At the study location *A.*

122 *percula* occupied two anemone species, *Stichodactyla gigantea* and *Heteractis magnifica*. We

123 compared local reproductive success for individuals breeding on the two different host species. We

124 found that offspring spawned by adults residing in *H. magnifica* and those from *S. gigantea*

125 recruited in similar proportions in the two species of anemone ($\chi^2=1.32$, P=0.250, Fig. 3*A*). There

126 was no tendency for individuals to recruit to their natal anemone species. However, local

127 reproductive success (*i.e.*, the likelihood of an individual to produce a self-recruiting offspring) was

128 always higher for individuals living in *S. gigantea* than for those living in *H. magnifica* (T=-2.41,

129 P=0.016, Fig. 3*B*). Hence, local reproductive success was dependent on which anemone species

130 larvae settled into, rather than which host anemone they came from. The largest extended families

131 at Kimbe Island were those in which offspring, perhaps by chance, settled on *S. gigantea* in each

132 generation. The geographic location of the anemone species (*i.e.* in shallow water and close to the

133 land for *S. gigantea* and in the lagoon for *H. magnifica*) may explain the difference observed

134 between the two anemones in contributions to self-recruitment. *Heteractis magnifica* is generally

135 more abundant further from shore than *S. gigantea* so may produce more dispersive larvae while *S.*

136 *gigantea* produces more self-recruiters.

Discussion

138 Our study revealed that consistently high levels of self-recruitment in the focal Kimbe Island orange

139 clownfish population resulted in longitudinal philopatry over five generations in a 10-year period.

140 The high self-recruitment rate observed is likely linked to biological characteristics of the orange

141 clownfish that include benthic spawning and relatively short pelagic larval duration (*i.e.* ~10 days,

142 (25)). Other factors, including local current patterns, may also influence the larval dispersal kernel

143 to some degree. Kimbe Island sits in a dynamic oceanic regime that is influenced by mesoscale

144 eddies, likely originating from instabilities in the South Equatorial Current and the New Guinea

145 Coastal Current (26), that may favor local retention (Fig. S2). Whatever the ultimate cause of the

146 high self-recruitment to Kimbe Island, we have demonstrated that it is possible to construct

147 genealogies in a clownfish population and test for effects of anemone species on local reproductive

148 success.

149 In most coral reef fish populations, dispersal acts to reduce the probability for inbreeding. When

150 self-recruitment is negligible, then the probability for inbreeding is also low. In the presence of

151 significant self-recruitment, we would expect a proportional frequency of inbreeding. While we

152   identified incidents of inbreeding *in A. percula,* it occurred at a very low frequency (>0.01%).

153   When dispersal is limited or natal philopatry is frequent, inbreeding may be avoided by mate choice

154   that excludes kin. Previous results from experiments conducted in aquaria on *A. percula* showed

155   that larvae do not discriminate between parents and unrelated individuals when choosing settlement

156   sites (27). The level of inbreeding in *A. percula* was much lower than in small, closed populations

157   of terrestrial animals where inbreeding depression results in a severe decline in genetic diversity

158   (23, 24). Although we found high levels of self-recruitment (~56%), the number of juveniles

159   arriving from other reefs appears to be sufficient to maintain high genetic diversity in the study

160   clownfish population. The two incidences of inbreeding that we observed may be due to the limited

161   number of anemones available for settlement at any given time (28) that suggests it will be

162   advantageous for settlers to remain at the first anemone they encounter (29). A dispersive larval

163   phase and a hierarchical breeding system likely compensates for the potential inability of

164   individuals to accurately identify and actively avoid mating with relatives. The hierarchical

165   breeding system is a size-based queue for the two breeding positions in an anemone that means it

166   can take up to 10 years to become a breeder (30); *i.e.* newly settled relatives that could engage in

167   inbreeding may never get the chance.

168   High levels of self-recruitment do not preclude long distance dispersal events in *A. percula*. Our

169   observation of 56% self-recruitment means that 44% of the population arrived as larvae from reefs

170   beyond Kimbe Island. Larvae that dispersed to the study population from other locations likely

171   represented a significant influx of genetic diversity. Therefore while the larval dispersal kernel may

172   be weighted towards self-recruitment, immigration nonetheless has a significant impact on the

173   genetic structure of the study clownfish population. It remains unknown whether local adaptation

174   can occur in this *A. percula* population. Locally adapted genes may be swamped by gene flow from

175   immigrants. However, evolutionary adaptive divergence and therefore local adaptation may occur

176   despite gene flow if selection is strong (31). The high levels of self-recruitment suggest local

177 adaptation is at least possible in *A. percula*. Identifying the possible extent, nature, and implications

178 of local adaptations represents an important area for future research.

179 Our findings have consequences for the management of spatially-structured marine fish populations

180 and the ability of future generations to adapt to environmental change. While it is well known that

181 exploited populations can be protected in marine reserves such as Kimbe Island, our study has

182 demonstrated that this benefit can ensure the protection of multiple generations. It is conceivable

183 that such long-term protection may prove futile if coral reef fish species are unable to cope with

184 rapid global climate change. Climate change may act to reduce the potential dispersal distance and

185 functional connectivity between populations of many species in oceans (27, 32). Philopatric

186 behavior may become the major source of local replenishment in the future. While inbreeding is

187 currently very low, the population may be at risk in the long term if immigration and hence genetic

188 diversity decrease. However, our discovery of five-generation family trees in the space of 10 years

189 suggests that the potential for local adaptation may be higher than previously thought in marine fish

190 populations. The presence of local adaptation would, in turn, provide a mechanism for population

191 resilience to climate-induced changes in environmental conditions.

192 **Materials and Methods**

193 **Study species and data collection.** Exhaustive sampling was carried out every two years from

194 2003 to 2013 on the entire orange clownfish (*Amphiprion percula*) population at Kimbe Island

195 (5°12'22.56" S, 150°22'35.58" E), West New Britain Province, Papua New Guinea. Here, *A. percula*

196 live in a mutualistic association with one of two host sea anemone species, *Stichodactyla gigantea*

197 and *Heteractis magnifica*. Within each clownfish group (*i.e.* clownfish within one anemone) of

198 typically 3-5 individuals, there is a size-based dominance hierarchy: the female is largest, the male

199 is second largest and the non-breeders rank progressively lower in the hierarchy as they decrease in

200 size. Bigger is the group, longer a settler has to wait to achieve reproductive status, so the lower the

201   likelihood of surviving to maturity (33). If the single female adult of a group dies, then the male

202   changes sex to female and the largest juvenile from the anemone becomes sexually mature as male.

203   At Kimbe Island, reproduction occurs year round, with females laying several hundred eggs in a

204   clutch near the pedal disk of the host anemone each lunar month. The eggs hatch after ~7 days of

205   paternal care into larvae that spend ~10 days (34) in the pelagic environment before settling on an

206   anemone that may or may not be on the natal reef (35).

207   The Kimbe Island population of *A percula* consists of 310 different anemones (176 *H. magnifica*

208   and 134 *S. gigantea*). Anemone positions were recorded with a GPS and depth was measured using

209   a dive computer. Anemones were surveyed during each bi-annual sampling period and all fish

210   within the anemones were counted, captured using hand nets, measured using calipers (total length

211   TL), fin-clipped underwater for genetic analysis. Each fish was processed on SCUBA *in situ*, with

212   each individual caught, processed next to the anemone and then released back on the same

213   anemone. The biggest fish in each anemone was identified as the female, the second largest

214   individual was assumed to be the male, and all other individuals were classified as subadult

215   (TL>35mm) or new-recruits (TL<35mm). The small pieces of fin tissue were preserved in 95%

216   ethanol in 2mL vials. The new-recruits were collected whole, and preserved in vials with 95%

217   ethanol.

218   We extracted DNA from a total of 4829 fin-clips (including 1192 new-recruits) following a

219   modified HotSHOT method (36) and subsequently stored the DNA at -20°C. We then screened the

220   DNA at 22 polymorphic microsatellite loci (20). We controlled for human errors and sample

221   contamination by using negative controls during PCR and electrophoresis. We also controlled for

222   genotype errors by re-amplifying and re-analyzing a sample of 789 individuals with the same typing

223   software (GeneMapper v3.7). The error rate was approximately 2%. Genetic diversity metrics such

224   as allelic richness, average expected and observed heterozygosities (H_S and H_O, respectively) and

225   the departure from Hardy-Weinberg equilibrium fixation index or inbreeding coefficient (F_{IS}) were

226   estimated by using the R package HIERFSTAT (37).

227 **Pedigree and family reconstructions.** Pedigree reconstruction was carried out by using the

228 genotype data from the parentage analysis. We first identified the individuals sampled multiple

229 times over the years by using the Excel macro GenAlex v6.5 (38) to compare multilocus genotypes

230 from the 2003, 2005, 2007, 2009, 2011 and 2013 samples. Juvenile fish were assigned to parental

231 pairs using software FaMoz (39). This software is based on the calculation of Log of the odds ratio

232 of putative parent-offspring pairs being true and determines critical thresholds to accept or reject

233 assignments by simulating true and false parent-offspring pairs. FaMoz does not require *a priori*

234 information of the proportion of candidate parents in the sample in order to determine critical LOD

235 thresholds (40). Overlapping generations were assumed for the pedigree construction to

236 accommodate the biology of the clownfish species (*i.e.*, reproduction frequency and lifetime), such

237 that two individuals within a cohort were defined as a potential parent for any individual sampled

238 the same year or the following years (Fig. 1*A*). We only retained offspring-parent-pair assignments

239 when genotypes were matched to fish living in the same anemone or in an anemone in close

240 proximity within the same year period (for more details see (20)). Because of the presence of

241 overlapping generations in this system, we rejected all assignments to single parents to avoid

242 possible false assignments to other related individuals that were not true parents. We accepted

243 offspring-parent-pair couples with LOD scores > 30.

244 We tested all the possible parent couple-offspring links between the different year-periods (Fig.

245 1*A*). First, we compared individuals sampled in the same year and we hypothesized that *(i)* two

246 adults from same or nearby anemone can be potential parents of subadults and new-recruits, *(ii)* two

247 adults from same or nearby anemone can be potential parents of other adults (*i.e.*, the data set of

248 offspring to test in FaMoz is exactly the same as the data set of parents). Second, we compared

249 individuals sampled in two different years and we hypothesized that *(iii)* two individuals from adult

250 or subadult categories living in same or nearby anemone and sampled at a specific year period can

251 be the parents of adults, subadults and new-recruits sampled at the following years, *(iv)* two

252 individuals from adult or subadult categories sampled in two consecutives sampling years can be

253   the parents of adults, subadults and new-recruits sampled the year periods following. We measured

254   the local reproductive success for each individual as the sum of self-recruiting offspring produced

255   from 2003 to 2013.

256   **Pedigree and dispersal analyses.** Pedigree summary statistics estimating pairwise relatedness

257   between all individuals and maximal depth pedigree were obtained by using the R package

258   pedantics (41).We constructed the pedigree containing only those individuals that were informative

259   (*i.e.*, a member of a family from Kimbe Island). Then, we analyzed separately the structure and

260   depth of each family that was present in the pedigree. The families were plotted as family trees

261   using the R package KINSHIP2 (42). Mothers are represented with a circle, males and subadults

262   with a square and new-recruits with a diamond. The same individual can be both a father and a

263   mother because of the sex changes through the life of the clownfish. This hermaphroditism has no

264   effect on the pedigree reconstruction. We used as representative symbol a circle within a square for

265   the individuals for which we detected sex change during the studied period.

266   Parentage analyses combined to sampling site information allowed us to identify offspring dispersal

267   from the natal anemone to the anemone of settlement. We evaluated if descendants preferentially

268   returned to the same anemone species (*H. magnifica* or *S. gigantea*) as their founder ancestors or

269   not. Chi-square test (χ^2) was used to evaluate if the anemone species of offspring was independent

270   of the anemone species occupied by parents. Then, we tested if the local reproductive success of

271   offspring was linked by the parental anemone species to evaluate a potential effect of parental

272   environment by using Student's test (T). Statistical analyses were performed using R version 3.0.2

273   (43) and their significance was interpreted with P=0.05.

274   **Acknowledgments**

278 research visa's were approved by the PNG government and research protocols were endorsed by the

279 Board of Mahonia Na Dari Research and Conservation Centre, Kimbe, PNG.

280 We thank the large number of volunteers who assisted in the field and collected tissue samples: R.

281 Brooker, S. Choukroun, P. Costello, J. Davies, D. Dixson, K. Furby, M. Giru, B. Grover, J. Hill, N.

282 Jones, K. McMahon, G. Nanninga, M. Noble, S. Noonan, N. Raventos Klein, M. Pinsky, M. Priest,

283 J. Roberts, J. Smith, T. Sinclair Taylor, N. Tolou, M. Takahashi, P. Waldie, M. White. Mahonia and

284 FeBrina provided essential logistic support. We are grateful to the traditional owners of the reefs

285 near Kimbe Island for allowing us access to their reefs.

286 Finally, we thank the two anonymous reviewers for their many insightful comments and their

287 contributions to the final draft.

Author contributions

289 S.P., G.P.J., and S.R.T. conceived the study; O.C.S., P.S.A., G.R.A., G.P.J., M.S., M.L.B., S.R.T.,

290 and S.P. collected field data; O.C.S. and P.S.A. carried out the molecular laboratory work and

291 parentage analysis; O.C.S. and B.P. carried out the analyses; O.C.S., J.A.M., B.P. and S.P. wrote

292 the first draft and all authors contributed substantially to revisions.

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391 **Figures**
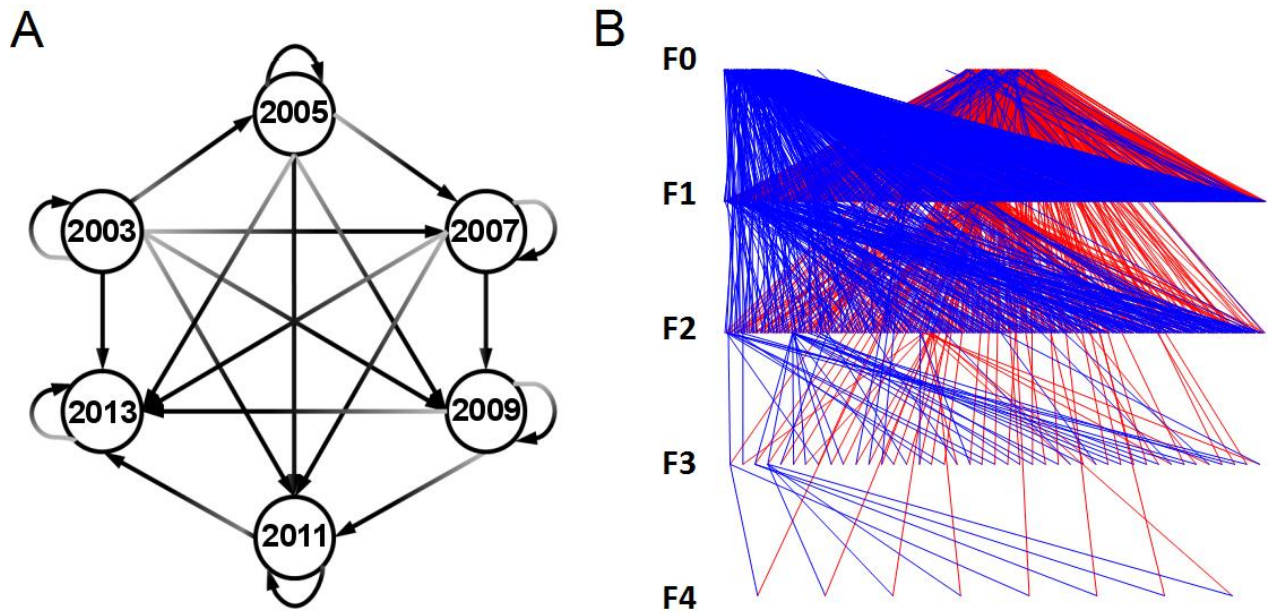
392
393 **Fig. 1.** Philopatric events found in the wild marine orange clownfish population of Kimbe Island

394 spanning over five generations. *(A)* Generational links among the six sampling periods (*i.e.*, 2003 -

395 '05,'07, '09, '11,'13) revealed by DNA parentage analysis. Orientation of the arrows gives the

396 generation direction (*i.e.*, from parent to offspring). *(B)* Pedigree representation - each line connects

397 a parent with one of its offspring (blue and red lines represent respectively paternal and maternal

398 links, an individual can be both mother and father). The generation of individuals is indicated on the

399 left from first generation (F0) to fifth generation (F4).
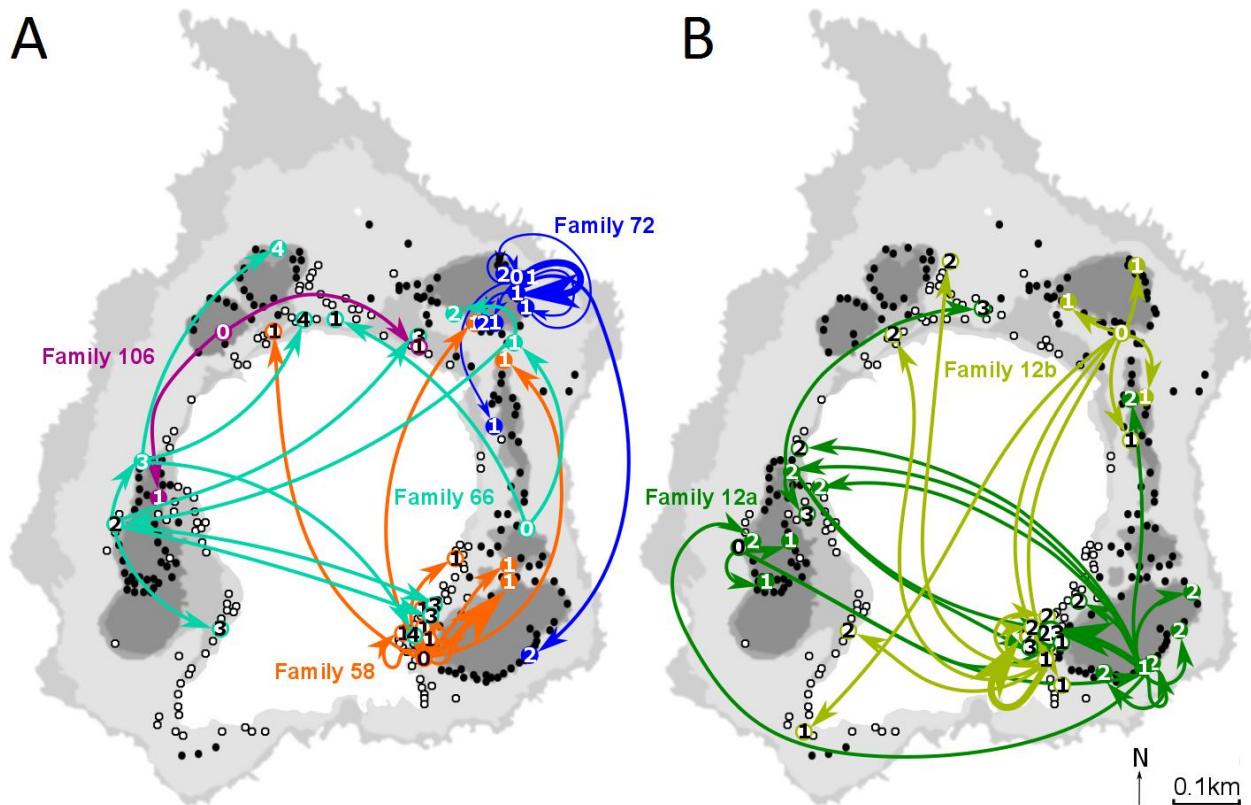
Fig. 2. Philopatric events in the wild marine orange clownfish population of Kimbe Island within and between microhabitats. Five representative families were selected to present the variation in size and depth of family trees (see Fig. S1). *(A)* Families spanning from 2 to 5 generations and *(B)* a family of 4 generations. Arrows represent links between parents and offspring. The direction of the arrow indicates the geographical origin and settlement of dispersal events. Dots refer to anemone locations (black and white dots correspond respectively to *Heteractis magnifica* and *Stichodactyla gigantea*). Numbers from 0 to 4 identify the corresponding generation in the pedigree. A dot with two numbers indicates the presence of related fish from different generations in the same anemone. White corresponds to the lands and shades of gray refer to the water, shallow water 0–2 m (light gray) and lagoons 2–15 m (dark gray).
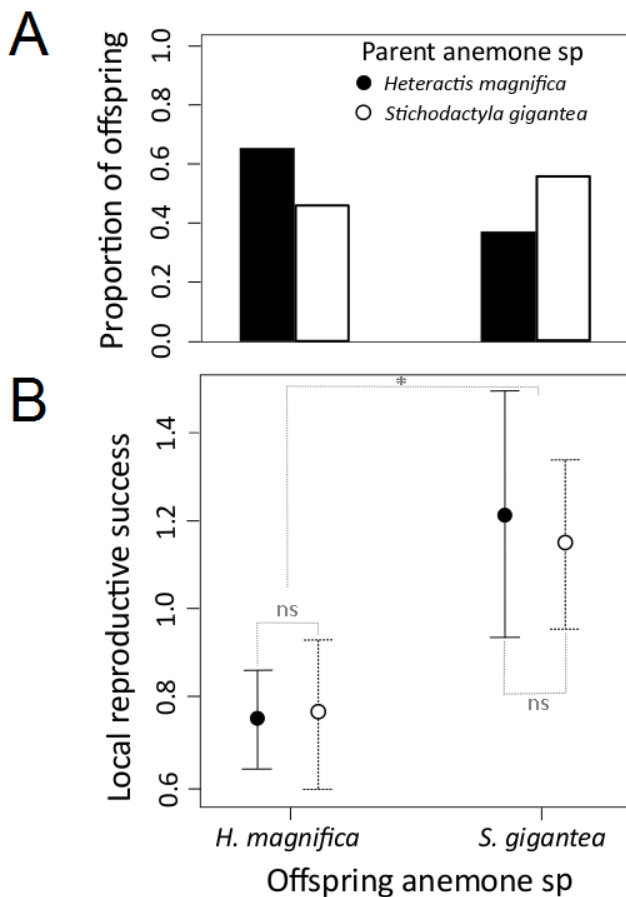
Fig. 3. Effect of parental anemone species on the local reproductive success of their offspring in

orange clownfish from Kimbe Island. *(A)* Settlement choice distribution of offspring (N=987

individuals) on the two species of anemones, *Heteractis magnifica* or *Stichodactyla gigantea*,

according to the anemone species of their parents. Offspring native from *H. magnifica* and those

native from *S. gigantea* recruited in similar proportion in the two species of anemone (χ^2=1.32,

P=0.250). *(B)* Effect of anemone species transmission from parent to offspring on the local

reproductive success of offspring. Whatever the anemone species from which the clownfish

progeny originated, the local reproductive success was always higher for individuals living in *S.*

gigantea than for those living in *H. magnifica* (T=-2.41, P=0.016). NS and * mean Student's

statistical test was not significant and significant, respectively.

Table

Table 1. Pedigree statistics and inbreeding in the Kimbe Island *Amphiprion percula* wild population. The number of individuals, families, founders and family links are listed in the central column. Total number of pairs of related individuals found within the same anemone are listed in the last column. Among these links, the number of cases of inbreeding are presented in brackets.

Pedigree statistic	Quantity	Pair of related individuals in anemone (number of inbred mating events)
N individuals	2927	57
N families	121	
N founders	502	
N maternal links	987	9
N paternal links	987	
N full sibs	1809	24 (1)
N maternal half sibs	412	2
N paternal half sibs	248	5
N maternal grandmothers	135	-
N maternal grandfathers	135	-
N paternal grandmothers	278	3 (1)
N paternal grandfathers	278	
N cousin	218	1
N uncle/aunt	308	10
N half uncle/aunt	165	2
N grand cousin	17	1
Pedigree maximum depth	4	