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Drivers of sociality in Gobiodon fishes: An assessment of phylogeny, ecology and life-history

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Abstract

What drives the evolution of sociality in animals? Many robust studies in terrestrial organisms have pointed toward various kinship-based, ecological and life-history traits or phylogenetic constraint which have played a role in the evolution of sociality. These traits are not mutually exclusive and the exact combination of traits is likely taxon-specific. Phylogenetic comparative analyses have been instrumental in identifying social lineages and comparing various traits with non-social lineages to give broad evolutionary perspectives on the development of sociality. Few studies have attempted this approach in marine vertebrate systems. Social marine fishes are particularly interesting because many have a pelagic larval phase and non-conventional life-history strategies (e.g. bi-directional sex-change) not often observed in terrestrial animals. Such strategies provide novel insights into terrestrially-derived theories of social evolution. Here, we assess the strength of the phylogenetic signal of sociality in the Gobiodon genus with Pagel's lambda and Blomberg's K parameters. We found some evidence of a phylogenetic signal of sociality, but factors other than phylogenetic constraint also have a strong influence on the extant social state of each species. We then use phylogenetic generalized least squares analyses to examine several ecological and life-history traits that may have influenced the evolution of sociality in the genus. We found an interaction of habitat size and fish length was the strongest predictor of sociality. Sociality in larger species was more dependent on coral size than in smaller species, but smaller species were more social overall, regardless of coral size. Finally, we comment on findings regarding the validity of the species G. spilophthalmus which arose during the course of our research. These findings in a group of marine fishes add a unique perspective on the evolution of sociality to the excellent terrestrial work conducted in this field.

Drivers of sociality in *Gobiodon* fishes: An assessment of phylogeny, ecology and life-history

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Highlights

- A phylogeny for the genus *Gobiodon* was inferred from seven molecular markers and resolved several previously unresolved nodes

- There is some evidence for a phylogenetic signal of sociality in the genus *Gobiodon*

- A combination of ecological and life-history factors best predicts sociality in the genus *Gobiodon*

- Phylogenetic analysis reveals potential issues surrounding the identification and validity of *Gobiodon spilophthalmus*

Abstract

What drives the evolution of sociality in animals? Many robust studies in terrestrial organisms have pointed toward various kinship-based, ecological and life-history traits or phylogenetic constraint which have played a role in the evolution of sociality. These traits are not mutually exclusive and the exact combination of traits is likely taxon-specific. Phylogenetic comparative analyses have been instrumental in identifying social lineages and comparing various traits with non-social lineages to give broad evolutionary perspectives on the development of sociality. Few studies have attempted

this approach in marine vertebrate systems. Social marine fishes are particularly interesting because many have a pelagic larval phase and non-conventional life-history strategies (e.g. bi-directional sex-change) not often observed in terrestrial animals. Such strategies provide novel insights into terrestrially-derived theories of social evolution. Here, we assess the strength of the phylogenetic signal of sociality in the *Gobiodon* genus with Pagel's lambda and Blomberg's *K* parameters. We found some evidence of a phylogenetic signal of sociality, but factors other than phylogenetic constraint also have a strong influence on the extant social state of each species. We then use phylogenetic generalized least squares analyses to examine several ecological and life-history traits that may have influenced the evolution of sociality in the genus. We found an interaction of habitat size and fish length was the strongest predictor of sociality. Sociality in larger species was more dependent on coral size than in smaller species, but smaller species were more social overall, regardless of coral size. Finally, we comment on findings regarding the validity of the species *G. spilophthalmus* which arose during the course of our research. These findings in a group of marine fishes add a unique perspective on the evolution of sociality to the excellent terrestrial work conducted in this field.

1.   Introduction

The question of how sociality first arose in animals has attracted much attention in the fields of

evolutionary ecology and animal behaviour. Many mechanisms are thought to contribute to the

evolution of sociality including ecological factors, life-history traits and phylogeny (Arnold and Owens,

1998; Emlen, 1982; Hamilton, 1964; Hatchwell and Komdeur, 2000; reviewed in Hing et al., 2017; Kokko

and Ekman, 2002). These features are not mutually exclusive and may be highly dependent on each

other (Arnold and Owens, 1998; Chapple, 2003). Hamilton's rule predicts that sociality should evolve

under certain combinations of relatedness and costs and benefits of social actions and is widely

regarded as a universal framework to study social evolution (Bourke, 2014; Hamilton, 1964). Ecology,

life-history and relatedness change the costs and benefits conferred to individuals within the group.

Under this framework, individuals should receive greater inclusive fitness benefits if they form social

groups with close relatives (Briga et al., 2012; Hughes et al., 2008). Groups consisting of unrelated

individuals are also possible if ecological or life-history factors alter the direct costs and benefits of

group living such that the benefits outweigh the costs (e.g. Buston et al., 2007; Riehl, 2011).

Phylogenetic relationships among taxa can constrain the evolution of sociality which may predispose

species to sociality (e.g. Agnarsson, 2002; Nowicki et al., 2018; Schneider and Kappeler, 2014;

Smorkatcheva and Lukhtanov, 2014). However, the extant state of sociality may depend on various

ecological and life-history conditions (Chapple, 2003; Rubenstein and Lovette, 2007; Schürch et al.,

2016). For example, altered environmental conditions and extreme weather events could reduce habitat

sizes for a normally social species, increasing animal density and increasing conflict within the group

ultimately leading to a reduction in sociality (Hing et al., 2018). On the other hand, some species in

which sociality has a strong phylogenetic signal (that is, sociality is highly constrained), may maintain

their sociality regardless of other factors (Kruckenhauser et al., 1999; Nowicki et al., 2018; Shultz et al.,

24 2011). In either case, understanding the strength of the relationship between phylogeny and sociality

25 can help us to understand what role phylogeny played in the evolution of sociality.

26 The majority of studies of sociality have been conducted on birds, mammals and invertebrates wherein

27 subordinates are usually related to dominants and display natal philopatry (Bourke, 2011; Hing et al.,

28 2017; Jennions and Macdonald, 1994; Jetz and Rubenstein, 2011; Rubenstein and Abbot, 2017). Habitat

29 specialist fishes on the other hand provide a unique opportunity to study social evolution as they often

30 reside in groups with low relatedness due to a pelagic larval phase (contrary to most terrestrial species;

31 Avise and Shapiro, 1986; Buston et al., 2007; but see Buston et al., 2009). In particular, coral gobies of

32 the genus *Gobiodon* are ideal for testing hypotheses about sociality as they display a wide variety of

33 social phenotypes (Thompson et al., 2007; Wong et al., 2007), are easily observed because they occupy

34 discrete habitat patches (Wong and Buston, 2013) and their phylogenetic relationships are reasonably

35 well established (Duchene et al., 2013; Hing et al., 2017).

36 Several previous studies have examined phylogenetic relationships among species of *Gobiodon*

37 (Agorreta et al., 2013; Duchene et al., 2013; Harold et al., 2008; Herler et al., 2009; Thacker and Roje,

38 2011). However, these studies have focused on relationships within the genus or more broadly at the

39 family level (Gobiidae). To date, no studies have investigated the phylogenetic patterns of sociality in

40 this genus. Duchene et al. (2013) examined the coevolution of *Gobiodon* species with their host corals

41 and provides the most recent and comprehensive phylogeny of the *Gobiodon* genus. Likewise, there

42 have been a number of studies investigating the causes and consequences of sociality in coral gobies

43 (*Gobiodon* and *Paragobiodon*), but these studies have often focussed on a single species or a subset of

44 species within the genus (Hing et al., 2018; Hobbs and Munday, 2004; Hobbs et al., 2004; Munday et al.,

45 2006; Thompson et al., 2007; Wong, 2010, 2011; Wong et al., 2007). Furthermore, no studies so far have

46 examined the relationship between sociality and ecological and life history traits across the genus

47 *Gobiodon* while controlling for phylogeny, and hence tested key hypotheses of social evolution.

48   In this study we resolved the phylogenetic relationships within the genus *Gobiodon* at Lizard Island

49   (Great Barrier Reef, Queensland, Australia) using seven molecular markers. Our reconstruction builds on

50   the inferred phylogeny of Duchene et al. (2013) by increasing the number of molecular markers used,

51   thereby inferring a phylogenetic tree with greater confidence. We then assessed the phylogenetic signal

52   of sociality in the genus. Given previous work on *Gobiodon* demonstrated plasticity in social organization

53   in response to extreme weather events (Hing et al., 2018), we expected to find a relatively weak

54   phylogenetic signal of sociality. However, we did not know *a priori* what the strength of the signal would

55   be and hence the extent to which shared evolutionary history of species would contribute to present

56   day patterns of sociality. We therefore tested a range of ecological and life-history characteristics with

57   phylogenetic structure in the models to assess the role these factors might have played in the evolution

58   of sociality in *Gobiodon.*

59   Previous studies have shown significant relationships between group size and the factors of habitat size

60   and body size in closely related species of coral gobies and more broadly in other species of habitat

61   specialist fish (*Amphiprion percula,* Buston, 2003; *Paragobiodon, Gobiodon* and *Eviota,* Thompson et al.,

62   2007; *Paragobiodon xanthosoma,* Wong, 2011). Most of the species in these previous studies form size

63   based social hierarchies and habitat size and body size have been shown to predict group size in these

64   species. A similar relationship has also been demonstrated between sociality and ecological generalism

65   in snapping shrimp (Brooks et al., 2017). Coral gobies are generally considered to be highly specialized in

66   their choice of corals (Munday et al., 1997). However, we observed considerable variation in coral

67   choice for some species, especially after extreme weather events (Hing et al., 2018). We also observed

68   some variation in social structure and therefore aimed to investigate whether a relationship existed

69   between sociality and host generalization. Hence, we specifically focused on two ecological variables: i)

70   host-coral size and ii) host coral generalization (the ability to inhabit a broad range of host coral species),

71   and one life-history variable iii) body size, and assessed their relationship with sociality.

72   Finally, we present findings on *Gobiodon spilophthalmus* concerning its phylogenetic placement, which

73   arose during our analyses. This is the first study to assess the phylogenetic basis and ecological and life

74   history correlates of sociality in *Gobiodon* and therefore provides an important starting point for

75   understanding the evolution of sociality in marine fishes.
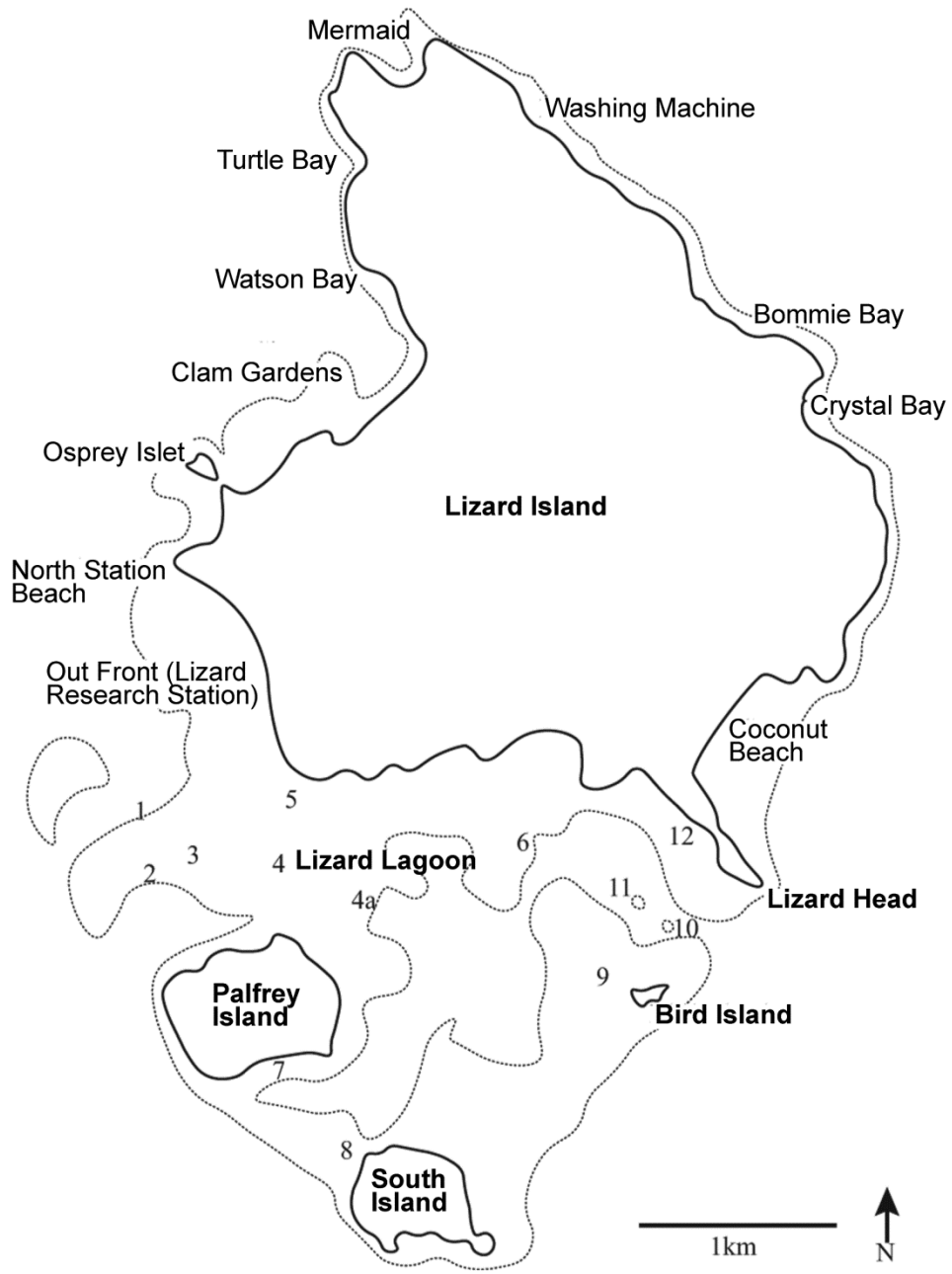
76   **2.   Methods**

77   *2.1.   Ethics approvals and research permits*

78   All research activities for this study were conducted with the approval of the University of Wollongong

79   Animal Ethics Committee (AE14-04, AE14-29). We conducted our research in the Great Barrier Reef

80   Marine Park under permits G13/36197.1 and G15/37533.1.

81   *2.2.   Field Sampling*

82   Tissue samples of fifteen species of *Gobiodon* were collected from 23 sites around Lizard Island between

83   February - March 2014 and January – February 2016 (Table 1, Fig 1). However, *G. spilophthalmus* was

84   removed from the analyses as barcoding analysis of the CO1 gene demonstrated the individuals

85   collected were likely juvenile specimens of *G. acicularis* and *G. ceramensis* (Section 3.4). We searched all

86   species of *Acropora, Stylophora, Seratopora and Echinopora* known to host *Gobiodon* fishes along 30 m

87   transects in the study area (Munday et al., 1999). Transects were placed haphazardly at each site and

88   only used as a reference to aid in the relocation of tagged corals (i.e. transects were not used for any

89   kind of spatial analysis). In total, 21 species of coral were recorded.

Map content labels:
Mermaid
Washing Machine
Turtle Bay
Watson Bay
Bommie Bay
Clam Gardens
Crystal Bay
Osprey Islet
Lizard Island
North Station Beach
Out Front (Lizard Research Station)
Coconut Beach
5
1
12
3
4 **Lizard Lagoon**
6
2
4a
11
Lizard Head
10
Palfrey Island
9
Bird Island
7
8
South Island
1km
N

90

Fig 1. Map of study sites at Lizard Island, Australia. Dotted lines indicate reef structure. Site names are in

regular font. Numbered sites are: Big Vickey's Reef (1); Vickey's Reef (2); Horse Shoe Reef (3); Palfrey

93   Reef (4 – 4a); Loomis Reef (5); Trawler (6); Picnic Beach (7); Ghost Beach (8); Bird Island Reef (9);

94   Entrance Bommie (10); Bird Bommie (11); Lizard Head Reef (12).

95

96   Corals were searched by divers with the aid of an underwater light for the presence of gobies. Corals

97   hosting gobies were identified to species and measured along three axes (length, width and height: Hing

98   et al., 2018). Gobies were removed from the corals by anesthetising them with a clove oil solution and

99   creating a current by hand (Munday and Wilson, 1997). The species and number (group size) of captured

100   fish was recorded and brought to a boat for processing. On the boat, fish were placed into a large

101   container of regularly refreshed seawater to maintain constant temperature and aeration. Each fish was

102   anesthetised and measured to the nearest 0.01 cm with vernier callipers and a small caudal fin clip (~1-2

103   mm) of each individual was preserved in ethanol. After processing, fish were released back to their

104   original coral of capture.

105   *2.3.   Ecological and life-history factors*

106   Coral size was calculated as the simple average diameter, (L + W + H)/3 as it provides a good

107   representation of the major axis of the coral (Kuwamura et al., 1994). Ecological generalisation was

108   assessed as the number of host-coral species each goby species was observed to occupy. We added

109   observations from three subsequent field trips between August 2014 and February 2016 for the

110   ecological generalisation analyses as two cyclones impacted the study site over this period (Hing et al.,

111   2018). We reasoned that these impacts had the potential to alter normal patterns of residency and

112   species adhering to a 'specialist' strategy would possibly broaden their host-species range under

113   extreme circumstances. We therefore wished to capture any variation these disturbances caused for

114   this analysis.

115 Body size was chosen as a life-history trait of interest for this study. We measured the standard length

116 (tip of the snout to caudal peduncle) of each individual. Standard length was used rather than total

117 length as many individuals had sustained damage to the caudal fin and an accurate measure of total

118 length could not be obtained.

119 *2.4.   Sociality index*

120 We used a sociality index proposed by Avilés and Harwood (2012). The index is an average for each

121 species, of the proportion of groups in the study population, proportion of subordinates in the study

122 population and proportion of the life-cycle spent in a group. The proportion of the life-cycle spent in a

123 group may be an important indicator of delayed dispersal in some species. However, coral gobies

124 undergo a pelagic larval phase prior to joining a group where they typically remain in a social queue to

125 obtain breeding status (i.e. they do not delay dispersal, but do tend to remain in a group once settled).

126 Therefore, we assumed the proportion of the life-cycle spent in the group was 1 for all species and the

127 main variation in sociality in coral gobies was caused by the remaining two components of the sociality

128 index. The proportion of groups in the study population is indicative of a species' tendency to form

129 groups, while the proportion of subordinates in the study population (associated with the proportion of

130 groups) is an indication of behaviour in terms of the subordinate's willingness to join a group and the

131 dominant member's willingness to tolerate them. The social index ranges from 0 to 1. Raw index values

132 were used in the Generalized Least Squares analyses (Section 2.9).

133 **Table 1.**

134 Goby species observed at Lizard Island with number of tissue samples obtained. Number of host-coral

135 species was used as a measure of host-generalization. Mean standard length (SL) and host-coral size (CS)

136 were calculated for each species.

Goby spp	Tissue Samples (n)	Coral species inhabited (n)	Mean SL (cm)	Mean CS (cm)
G. acicularis	3	1	1.91	55.20
G. aoyagii[†]	3	2	2.49	26.41
G. axillaris	3	4	3.09	23.76
G. brochus	4	9	2.54	16.50
G. ceramensis	6	2	2.69	27.23
G. citrinus	3	3	2.79	91.49
G. erythrospilus	3	11	2.60	23.31
G. fuscoruber[††]	4	10	2.75	29.95
G. histrio	3	10	2.80	23.22
G. oculolineatus	3	9	2.44	23.86
G. okinawae	3	11	2.12	43.95
G. quinquestrigatus	6	11	2.49	21.33
G. rivulatus	3	8	1.65	21.70
G. spilophthalmus c.f.[‡]	6	-	-	-
G. species D	3	1	2.84	27.33
P. xanthosoma	1	1	1.72	26.53

[†] *G. aoyagii* was previously referred to as *G. species A* as a placeholder but has now been formally

described by Shibukawa et al. (2013).

[††] *G. unicolor* (sensu Munday et al., 1999) was reassigned as *G. fuscoruber* by Herler et al. (2013).

[‡] Measurements of ecological and life-history factors were not obtained for *G. spilophthalmus c.f.* as

they were determined to be juveniles of other species and excluded from analyses.

2.5.   DNA extraction, amplification and sequencing

143   DNA was extracted from fin clips for two to three individuals of each species of *Gobiodon* and one

144   individual *Paragobiodon xanthosoma* which was used as an outgroup to the *Gobiodon* genus (Table 1).

145   We used a standard Proteinase-K salting out procedure to extract DNA (Aljanabi and Martinez, 1997).

146   DNA was resuspended in 20-50 µl of TE solution (1 mM Tris-HCl, 0.1 mM ethylenediaminetetraacetic

147   acid [pH 8]) and stored at 4 °C. We amplified nuclear recombination activating gene 1 (RAG1), nuclear

148   zinc finger protein of the cerebellum 1 (ZIC1) and the mitochondrial cytochrome c oxidase subunit 1

149   (CO1) gene using generic fish primers for each gene (primer sequences available in Supplementary Table

150   S1; Holcroft, 2005; Li et al., 2007; Ward et al., 2005 respectively). Where weak amplification occurred,

151   goby specific primers were designed using an alignment of the appropriate gene region made up of

152   sequences obtained from species which showed strong amplification (Supplementary Table S1).

153   Polymerase Chain Reactions (PCRs) were performed using MyTAQ Polymerase (Bioline, Australia) in

154   accordance with the manufacturer's instructions. The PCR conditions consisted of 2 minutes at 95 °C, 35

155   cycles of 1 minute at 94 °C, 1 minute at 45 - 65 °C (optimised for each gene and species), 1 minute at 72

156   °C and a final elongation of 5 minutes at 72 °C. PCR products were checked for length and strength of

157   amplification using 1% agarose gel electrophoresis. ExoSAP-IT (GE Healthcare, Bucks, UK) was used to

158   treat each PCR product prior to sequencing using the ABIPRISM BigDye Terminator v3.1 Cycle

159   Sequencing Kit (Applied Biosystems, Australia). Each PCR product was sequenced in both the forward

160   and reverse direction.

161   *2.6.   Sequence Alignment*

162   Alignment of RAG1, ZIC1 and CO1 genes was trivial, because there were no internal indels in the

163   alignment – both ClustalW and MUSCLE (within MEGA7; Kumar et al., 2016) produced alignments with

164   only leading and trailing gaps, where the length of reliable sequence was slightly different. The default

165   settings for both ClustalW and MUSCLE were used.

166   Once COI, RAG1 and ZIC1 sequences had been obtained for 2 to 3 individuals of each species, consensus

167   sequences were established using Bioedit (Hall, 1999). We then constructed additional consensus

168   sequences for 12S and 16S rRNA genes (obtained from GenBank, accession numbers available in

169   Supplementary Table S2) for the species in our study and obtained further consensus sequences for the

170   nuclear ribosomal protein S7 Intron 1 chromosome 2 (S7I1) gene and mitochondrial cytochrome *b* (cytb)

171   from GenBank (Supplementary Table S2; Duchene et al., 2013; Harold et al., 2008; Herler et al., 2009).

172   All seven genes (RAG1, ZIC1, S7I1, COI, cytochrome *b*, 12S and 16S) were concatenated for each species.

173   *2.7.   Phylogenetic analysis*

174   Partitioning schemes and nucleotide substitution models were established with PartitionFinder version

175   1.1.1 (Lanfear et al., 2012) using the corrected Akaike Information Criterion (AICc) and a heuristic search

176   algorithm with branch lengths unlinked. We performed the analysis on 7 datablocks, one for each gene.

177   Priors for the branching process and times were set as follows: the tree prior was a Yule model, the birth

178   rate had a uniform prior, as did the clock rates for each of the gene partitions. A strict clock was set for

179   each partition, but the clock rate was unlinked between partitions. Phylogenetic trees were then

180   inferred from Bayesian analysis conducted on BEAST2 v2.4.2 (Bouckaert et al., 2014; Drummond et al.,

181   2012) in which unlinked partitions and a Markov Chain Monte Carlo (MCMC) process with a chain length

182   of 100 million was specified. No calibration information was used as we only wished to examine relative

183   estimates of branching times. Separate BEAST analyses were also conducted on the concatenated

184   mitochondrial data (since the mitochondrial genes represent a single, linked locus), and each nuclear

185   gene fragment. These trees are reported in the supplementary material. The trees recovered from the

186   individual nuclear gene analyses were generally poorly resolved, with many nodes having low posterior

187   probability support. This is not surprising given the relatively small size of these datasets. The

188   mitochondrial tree was well resolved (with high posterior probability support), but differed in the

189   placement of one clade (i.e. Fig 3, clade B) when compared with the 'full data' set. We focus here on the

190   'full data' set, because it is larger and contains information from multiple (mitochondrial and nuclear)

191   sources.

192   Stationarity was assessed with Tracer v1.6 (Rambaut et al., 2018). In initial BEAST analyses, stationarity

193   was not reached after 100 million generations (expected sample sizes (ESS) values generally less than

194   200), primarily because some parameter values were very close to zero. However, when the nucleotide

195   substitution model for 6 of the 7 gene partitions was simplified (from GTR to HKY; in one of the gene

196   partitions, PartitionFinder suggested JC69, and this was kept as JC69), stationarity was reached after 100

197   million generations, with all EES values greater than 200. A maximum likelihood analysis was also

198   conducted using "Randomized Axelerated Maximum Likelihood" (RAxML) version 8 (Stamatakis, 2014).

199   The Gamma model of rate heterogeneity was used with branch lengths optimized per gene and the

200   proportion of invariable sites estimated. A maximum likelihood search was then applied to find the best

201   scoring tree.

202   *2.8.   Phylogenetic signal*

203   Phylogenetic signal of sociality was calculated in R using the phylosig() function of the phytools package

204   (Revell, 2012). We used the social index for each species and the Bayesian summary tree for the

205   analyses. We calculated both Pagel's lambda (Pagel, 1999) and Blomberg's K (Blomberg et al., 2003)

206   statistics and produced tests against a null hypothesis of no phylogenetic signal using a likelihood ratio

207   test and randomization test respectively.

208   *2.9.   Phylogenetic Generalized Least Squares models*

209   Phylogenetic Generalized Least Squares was used to assess relationships between sociality and

210   ecological and life-history traits while taking into account phylogenetic non-independence between

211   species. Sociality index was the dependent variable and the ecological and life-history traits were

212   included as main and interacting effects. We used a summary of the Bayesian inferred phylogenetic tree

213   for this analysis. All pGLS analyses were conducted using the nlme package in R (Pinheiro et al., 2018).

214   Four models of trait evolution (Brownian motion, Pagel's Lambda, Blomberg ACDC and Ornstein-

215   Uhlenbeck) available in the ape package (Paradis et al., 2004) were applied to each of the relationships.

216   As we had no *a priori* expectations of the type of selection sociality might be under, we chose the best

217   model to present by comparing Akaike's Information Criterion (AIC). An analysis of deviance was

218   conducted using the Car package (Fox and Weisberg, 2011) on the best model to identify factors that

219   significantly deviated from the null model.

220   *2.10.   Gobiodon spilophthalmus*

221   *Gobiodon spilophthalmus* was first described by Fowler (1944). However this description was based

222   upon a single preserved specimen. We therefore based our identification on Munday et al. (1999) who

223   provide a live specimen photo and describe *G. spilophthalmus* as uniform black in colour and only

224   distinguishable from *G. ceramensis* (also uniform black as adults) in the juvenile phase. The juveniles of

225   *G. spilophthalmus* are white with black stripes along the body and black spots on the head (Fig 2 (i)). We

226   collected specimens morphologically similar to those depicted in Munday et al. (1999) as *G.*

227   *spilophthalmus.* During collection, we noted a small *G. ceramensis* changed colour upon capture from

228   uniform black to the black and white stripes and spots similar to that described for juvenile *G.*

229   *spilophthalmus*. This was observed again in 2019 by colleagues at One Tree Island, Australia (Froehlich

230   pers. comm.; Fig 2 (v)). These observations prompted a closer examination of our *G. spilophthalmus c.f.*

231   specimens. *G. spilophthalmus c.f.* specimens were found on the coral species *Seriatopora hystrix* and

232   *Echionopora horrida* which are also inhabited (almost exclusively) by *G. ceramensis* and *G. acicularis*

233   respectively (Fig 2 (iii, iv) photos). Other *G. spilophthalmus c.f.* specimens were sometimes observed

234   associating with groups of *G. ceramensis* or *G. acicularis.* To investigate this further, we sequenced the

235   barcoding region (COI) of individuals resembling *G. spilophthalmus* from independent colonies of *S.*

236   *hystrix* and *E. horrida*, and compared them with individuals of *G. ceramensis* and *G. acicularis*. First, we

237   conducted an Automatic Barcode Gap Discovery (ABGD) analysis which groups COI sequences into

238   hypothetical species based on automatic detection of the 'barcode gap', the natural break in sequence

239   divergence that occurs when within-species divergence is compared to between-species sequence

240   divergence (Puillandre et al., 2012). We used the default settings and Kimura 2-P (K80) distances. We

241   then conducted a Bayesian phylogenetic analysis of the COI gene of *G. acicularis, G. ceramensis* and *G.*

242   *spilophthalmus c.f.* using BEAST2. In this analysis we coded each individual with the species of coral it

243   was collected from. We used the same methods described above (sections 2.6 and 2.7) for sequence

244   alignment and Bayesian analysis to infer a gene tree for this species group using *G. okinawae* as an

245   outgroup. Furthermore, *Gobiodon heterospilos* is described as similar in appearance to *G.*

246   *spilophthalmus* but lacking the black body stripes (presumably in the juvenile phase; Munday et al.,

247   1999). Steinke et al. (2017) deposited three COI sequences on the BOLD database for *G. heterospilos*

248   from Lizard Island, however the photo attached to the only juvenile in their collection (BOLD record

249   LIFS847-08) clearly possesses black body stipes. We therefore conducted a second Bayesian

250   phylogenetic analysis using the same methods described above (sections 2.6 and 2.7) of our specimens

251   of *G. acicularis, G. ceramensis*, *G. spilophthalmus c.f.* and the *G. heterospilos* sequences deposited by

252   Steinke et al. (2017) in order to determine if *G. heterospilos c.f.* could be differentiated from species

253   identified in our collection.

(i)

© Roger Steene

(ii)

Gobiodon
heterospilus
SR3

Steinke et al. (2017)

(iii)

(iv)

(v)

Catheline Froehlich

254

255 **Fig 2.** *Gobiodon spilophthalmus* as depicted by Munday et al. (1999) (i) and *G. heterospilos* sample

256 deposited by Steinke et al. (2017) on the BOLD database, record LIFS847-08 (ii). Specimens from our

257 collection matching descriptions of juvenile *G. spilophthalmus* collected in 2014 from *Seriatopora hystrix*

258 (ii) and *Echinopora horrida* (iv). A small *G. ceramensis* transitioning from the suspected juvenile spots

259 and stripes pattern to the uniform black adult phase (v).

260

261 **3.   Results**

262 Our results suggest a combination of ecological and life-history factors contributed to the evolution of

263 sociality in the *Gobiodon* genus, but sociality by itself also has some evidence of a phylogenetic signal.

264 Phylogenetic analyses by two methods inferred identical species composition of four clades giving high

265 confidence in the phylogenetic tree used for pGLS analyses. Phylogenetic generalized least squares

266 analyses then demonstrated coral size and mean body size of the species likely have a strong influence

267 on the extant social state of a species (Section 3.3).

268 *3.1.   Phylogenetic inference*

269 Both analyses; Bayesian and maximum likelihood, produced four clades (A-D; Fig 3) containing exactly

270 the same *Gobiodon* species within each clade. The main difference between both analyses was the

271 Bayesian tree inferred 2 main sister groups (A/B and C/D sister clades) with strong support (posterior

272 probability 1.00) while the maximum likelihood tree was unresolved at the base of each sister clade

273 (bootstrap support <50). However it still produced the same 4 clades with the same configuration. The

274 two main sister groups inferred with the Bayesian tree each in turn formed two sister clades: clade A

275 and B with moderate support (posterior probability 0.79) and the sister clades of C and D with strong

276 support (posterior probability 0.99). Clade A resolved *G. acicularis* and *G. ceramensis* as sister species

277   (posterior probability 1.00), and contained two other species, *G. okinawae* (posterior probability 1.00)

278   and *G. citrinus* (posterior probability 1.00) (Fig 3). The species *G. oculolineatus, G. quinquestrigatus, G.*

279   *species D* and *G. rivulatus* made up clade B with *G. quinquestrigatus* and *G. species D* as sister taxa

280   (posterior probability 1.00) (Fig 3). Clade C contained a single sister species group made up of *G. aoyagii*

281   and *G. brochus* (posterior probability 0.99) (Fig 3). Clade D contained two sister species groups, the first

282   consisting of *G. histrio* and *G. erythrospilus* (posterior probability 1.00) and the second consisting of *G.*

283   *fuscoruber* and *G. axillaris* (posterior probability 1.00) (Fig 3).
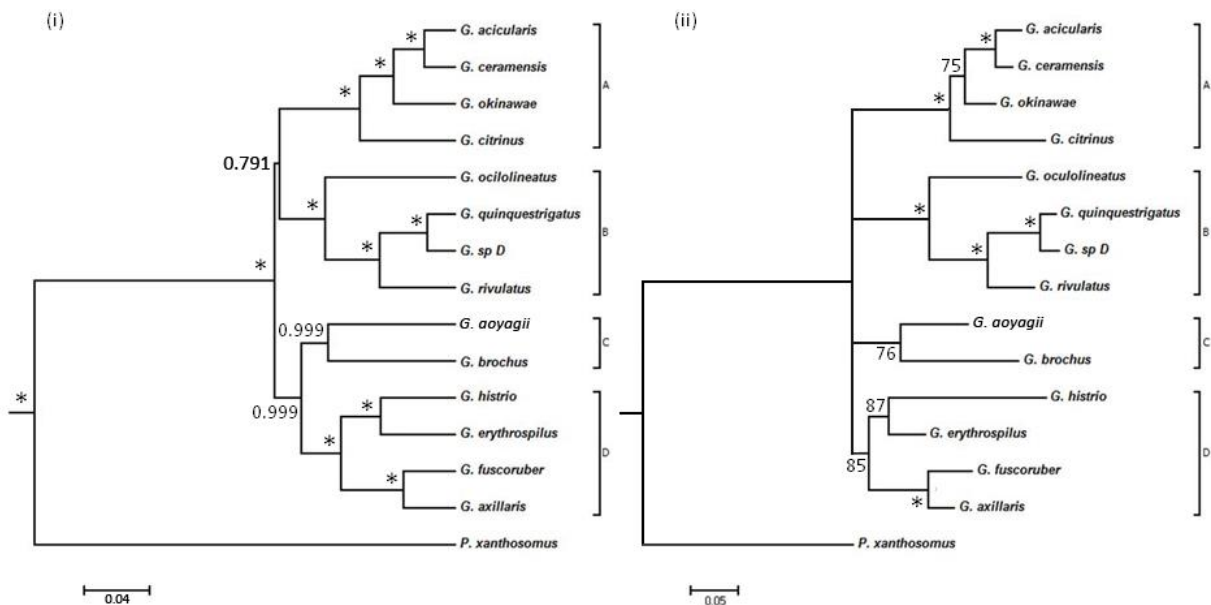
284

285   **Fig 3.** Phylogeny of *Gobiodon* present at Lizard Island based on 7 molecular markers (4 mtDNA; COI,

286   cytb, 12S, 16S and 3 nuclear DNA; RAG1, ZIC1, S7I1) produced with Bayesian (i) and maximum likelihood

287   (ii) methods. Node values in (i) are posterior probability where * indicates a value of 1. Node values in

288   (ii) are bootstrap percentages where * indicates a value of 100.

289

290   In the maximum likelihood analysis, the node giving rise to the A/B/C group could not be resolved with

291   any certainty (bootstrap support <50). However the configuration of the species within each clade was

292   identical to the Bayesian analysis and resolved with moderate to strong bootstrap support (75 – 100).

293   The strong support for the nodes within each clade in both analyses signifies reasonable confidence in

294   the species composition of each clade. The Bayesian analysis produced a tree with very high posterior

295   probabilities (with the exception of the node relating clades A and B). We therefore based all further

296   analyses on the Bayesian analysis.

297   *3.2.   Phylogenetic signal*

298   There was some evidence of a phylogenetic signal of sociality in the *Gobiodon* genus. We found little

299   evidence of a phylogenetic signal of sociality in the genus using Pagel's lambda (λ = 0.614, P = 0.349).

300   However, Blomberg's *K* displayed some evidence of a phylogenetic signal of sociality (*K* = 0.802, P =

301   0.035). Although the value of *K* represents a relatively low signal, the significant test result indicates it

302   was stronger than expected under a random distribution of the trait (sociality).

303   *3.3.   Phylogenetic generalized least squares*

304   There was a significant interaction between coral size and mean fish length in the pGLS model predicting

305   sociality (analysis of deviance, df = 1, χ^2 = 4.845, λ = 1.043, P = 0.028). The model predicted coral size

306   would have little impact on sociality for smaller species, but smaller species would generally be more

307   social (social index approximately 0.75, Fig. 4). On the other hand, sociality in larger species was much

308   more dependent on host-coral size (Fig 4). In other words, smaller species overall are predicted to be

309   more social than larger species regardless of the size of coral they inhabit, whereas larger species are

310   predicted to exhibit sociality only when corals are large.
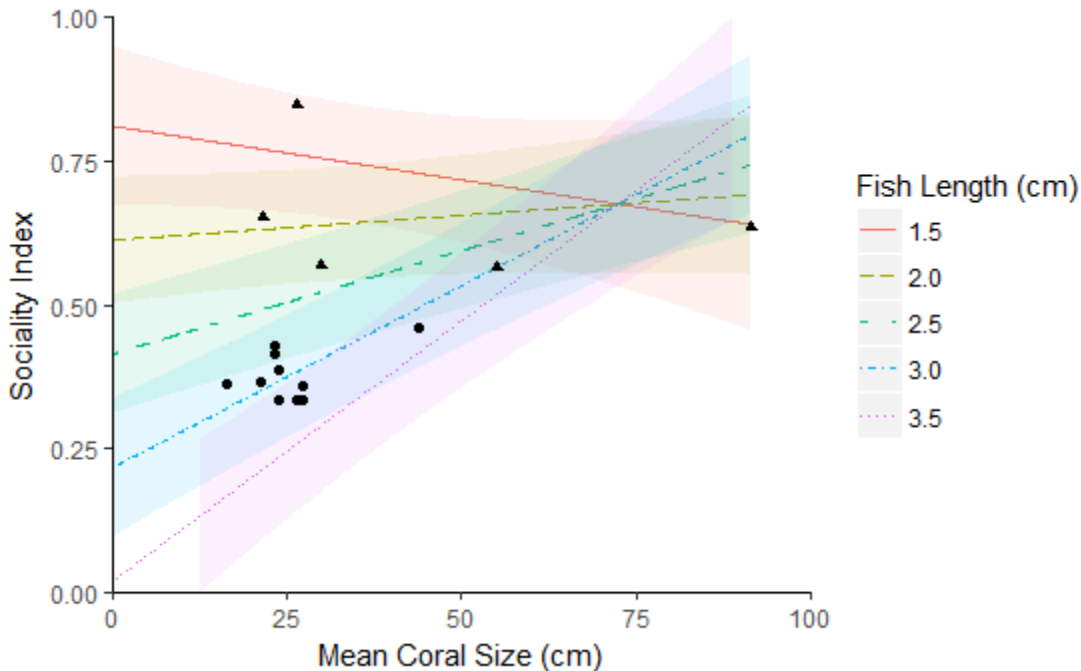
311

312   **Fig 4.** Model predictions for the interacting effects of host-coral size and fish length on sociality index.

313   Raw data are pair-forming species (circles) and group-forming species (triangles). Modelled species

314   sizes, indicated by different line types (figure legend), range from 1.5 cm (solid) to 3.5 cm (dotted).

315

316   There were no significant interactions between coral size and host generalization or mean fish-length

317   and host generalization on sociality in the respective models (df = 1, χ^2 = 0.781, λ = 1.073, P = 0.377; df =

318   1, χ^2 = 0.024, λ = 1.073, P = 0.878 respectively, Fig 5). This means there was no significant difference in

319   the relationship between sociality and host-coral size between species that adhere to either specialist or

320   generalist host strategies. Likewise, there was no significant difference in the relationship between

321   sociality and fish-length between host-specialist and -generalist species. The main effect of host

322   generalization alone was also non-significant (df = 1, χ^2 = 0.063, P = 0.803) indicating that the ability to

323   occupy a greater host-range is not likely to facilitate sociality in these species.

324
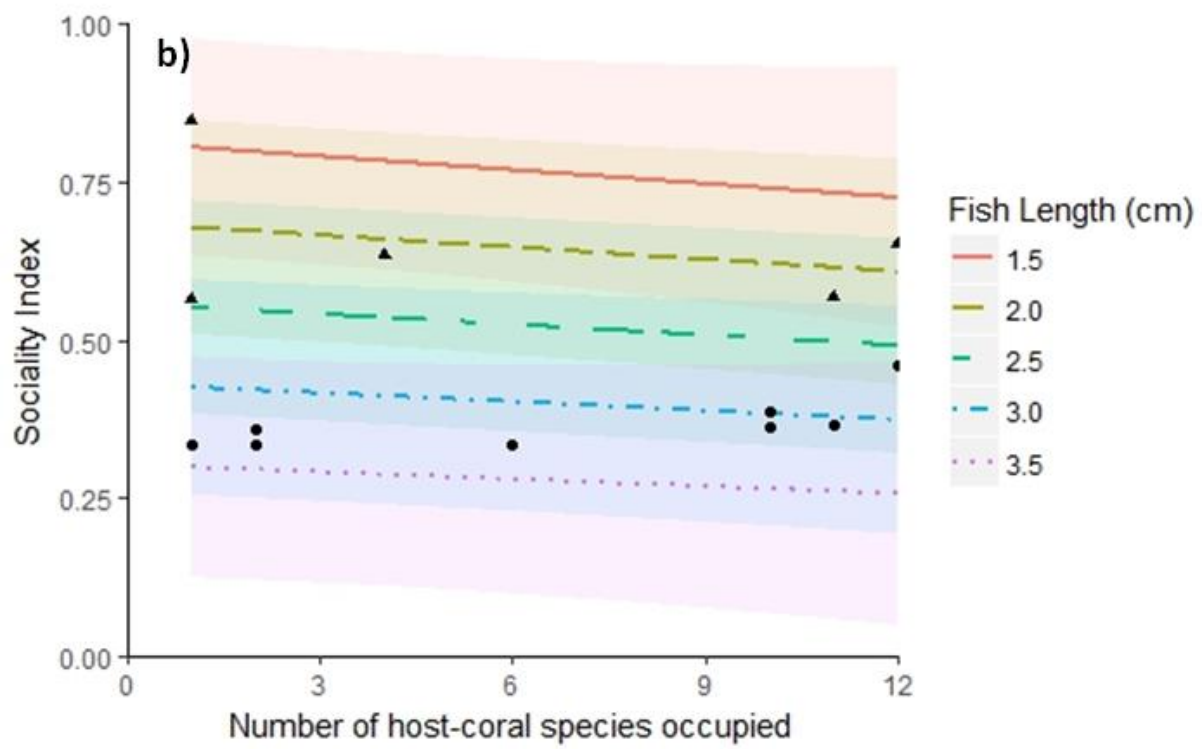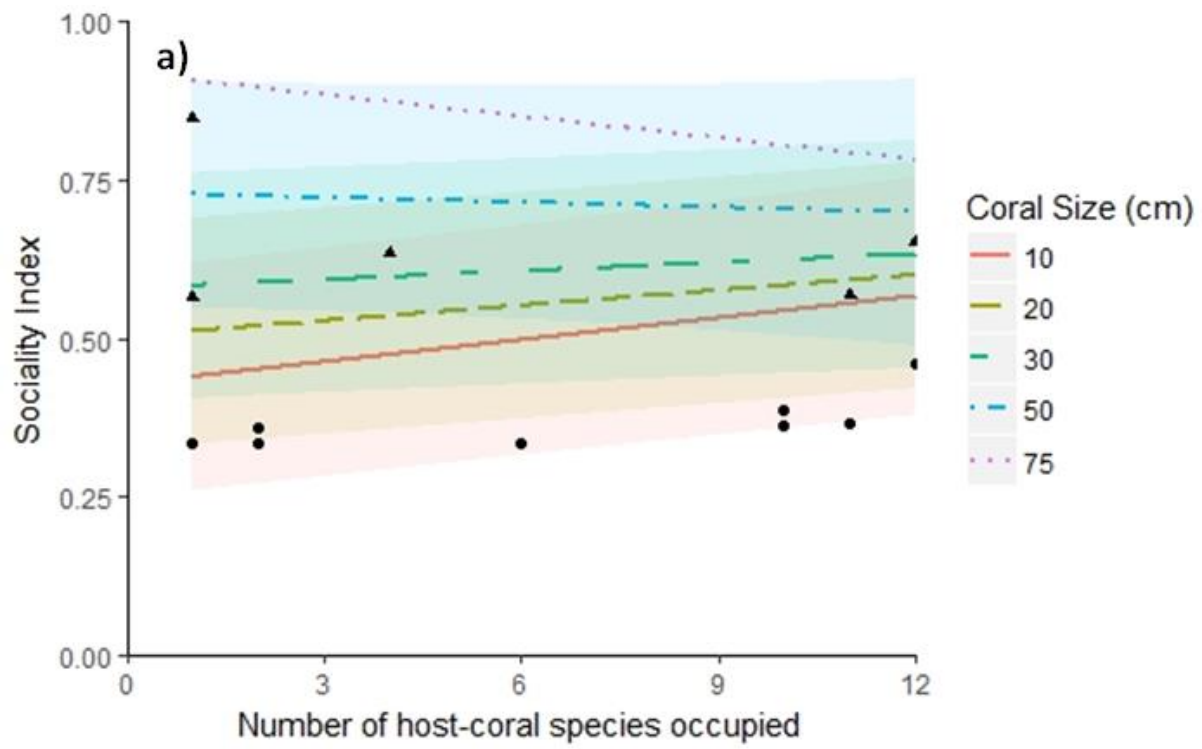
325 **Fig 5.** Interacting effects of mean coral size and host-generalization (a) and mean fish length and host-

326 generalization (b) on sociality. Lines in a) are different average coral sizes from 10 cm (solid line) to 75

327 cm (dotted line). Lines in b) are different mean fish length from 1.5 cm (solid line) to 3.5 cm (dotted

328 line). Both (a) and (b) raw data are individual species conforming to group-forming (triangles) or pair-

329 forming (circles) strategies.

330

331 While the detection of a phylogenetic signal of sociality was somewhat unconvincing in the test of

332 Pagel's Lambda and Blomberg's *K* (Section 3.2), the pGLS analyses showed a strong indication of

333 phylogenetic signal ($\lambda > 1$). Taken together these results indicate there is some phylogenetic signal of

334 sociality, but other effects (such as ecology and life-history) are probably equally, if not more important

335 in determining the extant social state of a species.

336 *3.4.   Gobiodon spilophthalmus*

337 Our analyses revealed the *G. spilophthalmus c.f.* specimens were likely juveniles of *G. acicularis* or *G.*

338 *ceramensis* depending on which coral species they were collected from. The ABGD analysis revealed two

339 distinct species groups, with the *G. spliophthalmus c.f.* specimens collected from *S. hystrix* grouping with

340 *G. ceramensis* and those collected from *E. horrida* grouping with *G. acicularis*. This pattern was also

341 supported in the Bayesian analysis of these COI sequences (Fig 6). This phylogeny showed *G.*

342 *spilophthalmus c.f.* grouping with both *G. ceramensis* and *G. acicularis*, depending on their respective

343 host corals. *Gobiodon ceramensis* did split into two groups in this analysis, but HKY distances ranged

344 from 0.2% to 0.7% indicating extremely low divergence in the COI sequences, a strong indication they

345 should be considered a single species. When we included the *G. heterospilos* sequences deposited by

346 Steinke et al. (2017) into a Bayesian phylogenetic analysis with our *G. spilophthalmus c.f., G. ceramensis*

347 and *G. acicularis* specimens, the *G. heterospilos* samples were placed in the same groups as *G.*

348   *spilophthalmus c.f.* (collected from *S. hystrix*) and *G. ceramensis* (posterior probability 0.999). We

349   therefore suspect Steinke et al. (2017) understandably misidentified these specimens in their study and

350   we did not include them in further analyses. These analyses indicate the specimens we collected, which

351   were morphologically similar to *G. spilophthalmus,* were most likely juveniles of either *G. ceramensis* or

352   *G. acicularis* and could be reliably differentiated by the species of coral they were collected from. We

353   therefore did not include *G. spilophthalmus* in our broader phylogenetic analyses.
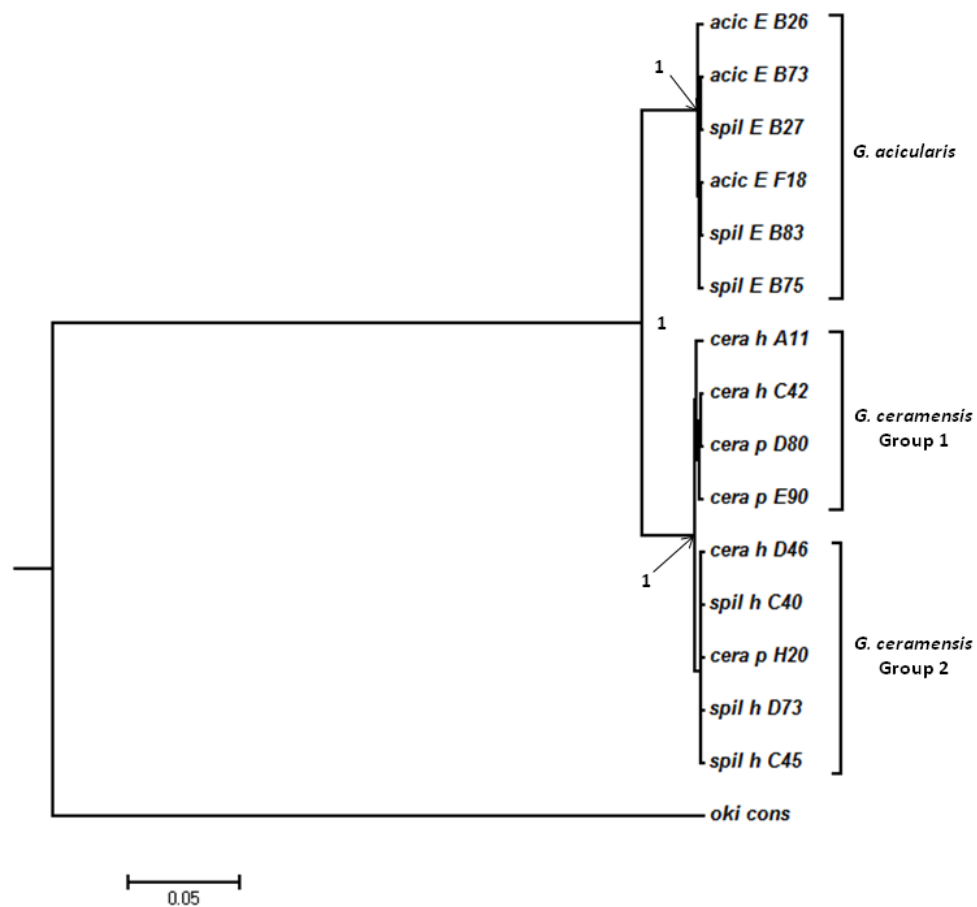
354

355   **Fig 6:** Phylogenetic tree produced with Bayesian analysis showing *G. acicularis* grouping with specimens

356   resembling *G. spilophthalmus*, and the two groups of *G. ceramensis* also recovered with specimens

357   resembling *G. spilophthalmus*. Node values are posterior probabilities. Values for internal nodes of each

358   species group are not displayed as the placement of individuals within each group is irrelevant. Species

359   names are abbreviated to acic (*G. acicularis*), spil (*G. spilophthalmus c.f.*), cera (*G. ceramensis*) and the

360   outgroup, oki (*G. okinawae*). Letters immediately following each species abbreviation indicates the coral

361   species the specimen was collected from; *Echinopora horrida* (E), *Seriatopora hystrix* (h) and *Stylophora*

362   *pistillata* (p). The last three characters are an individual identifier. The outgroup was a consensus

363   sequence (cons) of the COI gene.

364

4.   Discussion

366   Our analyses provide evidence of some phylogenetic signal of sociality in the coral-gobies, *Gobiodon*. In

367   contrast to several other vertebrate groups which display strong phylogenetic signals of sociality, our

368   findings suggest factors such as ecology, life-history or both, likely have a stronger impact on which

369   species display sociality at any given time (Kruckenhauser et al., 1999; Nowicki et al., 2018; Shultz et al.,

370   2011). In support of this, Hing et al. (2018) showed the mean group size of social species of *Gobiodon*

371   displayed plastic responses following multiple major ecological disturbances, suggesting sociality may be

372   quite flexible in *Gobiodon* species rather than phylogenetically constrained.

373   While Hing et al. (2018) did not delve into any species-specific trends, it is possible the observed social

374   plasticity was driven by a few key species (e.g. *G. acicularis, G. erythrospilus, G. fuscoruber, G. histrio* and

375   *G. okinawae*). These particular species have social indices close to 0.5 (the value exactly half-way

376   between theoretically perfect sociality and completely solitary) because there was a relatively even

377   proportion of groups and pairs in the study population (Hing et al., 2018). This indicates a certain level of

378   social plasticity in these species – when conditions allow, they will form groups, but they are also able to

379   survive as a breeding pair. These species are therefore prime candidates for further study of social

380   plasticity.

381   Like many cryptobenthic fishes, *Gobiodon* species have a pelagic larval phase where the larvae are

382   mixed with other nektonic organisms (Brandl et al., 2018). It therefore seems likely that relatedness

383   within the group would be low, as for other marine fishes (Avise and Shapiro, 1986; Buston et al., 2007;

384   but see Buston et al., 2009), although this is yet to be empirically tested. Low relatedness reduces the

385   value of 'r' in Hamilton's rule and hence the likelihood of sociality evolving, all else being equal (Bourke,

386   2014; Hamilton, 1964). For sociality to evolve in such groups, there must therefore be other factors

387   which alter the direct costs and benefits of group living. This was recently demonstrated in freshwater

388   cichlids by Dey at al. (2017) who found direct benefits provided from group living, biparental care and

389   diet type, were more influential than relatedness (associated with social monogamy) in the evolution of

390   cooperative breeding, a complex form of sociality. This contrasts with many other vertebrate lineages

391   which often form groups of closely related individuals and in which indirect (kin) benefits are likely to

392   have heavily influenced the evolution of social groups (Bourke, 2014; Halliwell et al., 2017; Lukas and

393   Clutton-Brock, 2012; While et al 2009; but see Riehl, 2013). This emphasis on direct costs and benefits

394   represents an alternate pathway to complex sociality to the kinship-based pathway often proposed in

395   the vertebrate literature. Alternatives such as this are worthy of further exploration as they offer novel

396   insights into the evolution of sociality (Dey et al. 2017; Riehl, 2013).

397   We tested factors known to provide direct fitness benefits in other closely related species, namely the

398   effects of host coral size, host coral generalization (ecological factors) and body size (life-history factor)

399   on sociality (Buston, 2003; Thompson et al., 2007; Wong, 2011). We found there was a significant

400   interaction between host coral size and body size on the degree of sociality when phylogenetic

401   correlation was accounted for. The relationship between host coral size and sociality was stronger for

402   lager species. This makes intuitive sense as individuals of larger species would presumably take up more

403   physical space in a coral. Hence, for larger bodied species to form groups, they would need to inhabit

404   larger corals on average. On the other hand, smaller species could potentially form larger groups in a

405   much larger size-range of corals before the habitat becomes saturated and group members are forced

406   to disperse from the group. Group sizes of various social fish species are not only influenced by habitat

407   size, however, and are instead related to size differences maintained between adjacent ranked

408   individuals (Mitchell & Dill, 2005; Buston et al. 2006; Ang & Manica 2010; Wong 2011). Thus, it is also

409   possible that smaller bodied species of *Gobiodon* maintain larger size ratios (smaller size differences)

410   between adjacent ranked group members than larger bodied species, which would be an important

411   avenue of future research.

412   Although smaller species showed less of a relationship between sociality and host-coral size, they were

413   more social overall than larger species. This may indicate that smaller species obtain greater direct

414   fitness benefits from social living or face greater constraints of dispersal or greater costs of solitary

415   living. For example, smaller species might be more prone to predation or less competitive for vacant

416   habitat compared to larger species, thus limiting dispersal opportunities and enhancing the benefits of

417   remaining within a group (Helfman and Winkelman, 1997; Munday and Jones, 1998). This finding is

418   again at odds with other terrestrial vertebrate systems which generally exhibit a positive relationship

419   between sociality and body size (Armitage, 1981; Bekoff et al., 1981). This discrepancy between

420   terrestrial and marine vertebrates highlights the importance of studying animal groups with varying life-

421   history strategies.

422   While host generalization has been proposed as a driver of sociality in some habitat specialist marine

423   species (Brooks et al., 2017), we found no evidence that it played a role in *Gobiodon* sociality. There was

424   considerable variation in the number of host-coral species inhabited by each species of *Gobiodon* but

425   this variation showed no discernable pattern in association with sociality. Munday et al. (1997)

426   demonstrated *Gobiodon* species have distinct coral preferences. However, our research suggests some

427   species appear to be more capable of relaxing this preference than others (especially during intense

428   ecological disturbance; e.g. Hing et al., 2018). This ability does not however, appear to be related to

429   sociality. The coral preferences displayed by many *Gobiodon* species may be due to properties of

430   particular coral species such as complexity, branch length or inter-branch distances (Untersteggaber et

431   al., 2014). Sociality might therefore be influenced by coral properties, not measured in this study rather

432   than variation in host-preference. For example more complex corals might increase the benefits of

433   remaining in the group (for example by offering greater protection from predators) and thereby

434   promote sociality. A similar pattern of increasingly complex habitat and a higher density of lizard

435   aggregations has been documented by Michael et al. (2010). Untersteggaber et al. (2014) demonstrated

436   that coral occupancy by *G. histrio* and *G. rivulatus* was related to coral size and branch length. Given our

437   findings on sociality and coral size, coral architecture would be an interesting factor to consider in future

438   studies of *Gobiodon* sociality.

439   To date, there have been few comparative studies of marine fishes looking at phylogenetic, ecological

440   and life-history correlates of sociality across multiple species (Hing et al., 2017; but see Nowicki et al.,

441   2018). In contrast, numerous studies in other vertebrate systems have been instrumental in developing

442   our current understanding of how ecology (Brown, 1974; Emlen, 1982; Kokko et al., 2002; Kokko and

443   Ekman, 2002; Stacey and Ligon, 1991) and life-history (Arnold and Owens, 1998; Hatchwell and

444   Komdeur, 2000; Rowley and Russell, 1990) have influenced the evolution of sociality in these systems

445   (reviewed in Hing et al., 2017). For example, phylogenetic reconstructions of sociality in other vertebrate

446   systems have revealed non-random clustering in birds and mammals (Arnold and Owens, 1998; Briga et

447   al., 2012; Edwards and Naeem, 1993). Closer examination at the genus level has revealed likely

448   ecological and life-history correlates of sociality (e.g. Armitage, 1981; Faulkes et al., 1997). We have now

449   added a comparatively understudied group of vertebrates with non-conventional life-histories (marine

450   fishes) to this knowledge base. Unconventional life-history strategies (such as bi-directional sex change

451   observed in several species of *Gobiodon*; Cole, 2011; Cole and Hoese, 2001; Munday et al., 1998;

452 Nakashima et al., 1996) likely alter the costs and benefits of group living in these social systems and

453 therefore represent a unique perspective on social evolution (Buston and Wong, 2014; Hing et al., 2017;

454 Wong and Buston, 2013).

455 *4.1.   Comparison of taxonomic structure*

456 We built upon the phylogeny of Duchene et al. (2013) by adding additional molecular markers. Our

457 Bayesian analysis inferred similar species composition (albeit with fewer species as we did not sample

458 from the Red Sea) of each clade to that of Duchene et al. (2013), but the placement of the clades

459 relative to each other varied between the two studies. Both studies inferred two sister species groups

460 with high posterior probability. However, the sister clades C/D in our study, inferred with strong

461 support, were not sister to each other in Duchene et al. (2013). Instead clade C was sister to cade A and

462 the other group consisted of clades B/D in Duchene et al. (2013). Our tree provides very strong support

463 for the sister group C/D while the node relating clades C and A in Duchene et al. (2013) is inferred with

464 moderate support. However the A/B group in our study was not strongly supported. It seems there is

465 broad agreement in the species composition of each clade. However, further research into the

466 relationships between the clades is clearly required to discern the true genetic structure of the genus.

467 *4.2.   Gobiodon spilophthalmus*

468 We determined our *G. spilophthalmus c.f.* specimens were in fact juveniles of either *G. ceramensis* or *G.*

469 *acicularis* depending on the host-coral they were collected from. To our knowledge this is the first

470 record of these species having juveniles of similar appearance to each other and to those described as *G.*

471 *spilophthalmus* (Fowler, 1944; Munday et al., 1999). Our findings raise several possibilities. First, *G.*

472 *spilophthalmus* may not be a valid species. The phylogeny produced by Duchene et al. (2013) shows very

473 low support for the node relating *G. spilophthalmus* to *G. ceramensis* indicating there was difficulty

474 delineating these samples as separate species. Harold et al. (2008) recognise *G. spilophthalmus* as a valid

475   species, but do not include it in their phylogeny of Indo-Pacific *Gobiodon* species. Second, *G.*

476   *spilophthalmus* could be a valid species but is not present at Lizard Island. We cannot rule this possibility

477   out with our data, but we find it unlikely that a species described from the New Hebrides (Vanuatu)

478   would not be present at Lizard Island especially given the broad distribution of its congeners (Fowler,

479   1944; Munday et al., 1999). Additionally, Munday et al. (1999) describe *G. spilophthalmus* as occurring

480   throughout the range of their collections which includes the Great Barrier Reef and Papua New Guinea.

481   Third, *G. spilophthalmus* is a valid species and is present at Lizard Island, but we did not sample any.

482   Although we sampled as many reefs as possible at Lizard Island, fourteen goby colonies may not be

483   representative of the whole Lizard Island population, especially if *G. spilophthalmus* is rare. Additionally,

484   there is clearly confusion around the identification of *G. heterospilos* and *G. spilophthalmus* in the

485   literature (Fowler, 1944; Munday et al., 1999; Steinke et al., 2017), assuming both are indeed valid

486   species as recognized by Harold et al. (2008).

487   Assuming *G. spilophthalmus* is a valid species, it appears to have diverged very recently and is therefore

488   very closely related to its sister species, *G. ceramensis* (Duchene et al., 2013). It is likely the genetic

489   markers used in our analysis (COI) and other studies featuring *G. spilophthalmus*, are evolving more

490   slowly than this clade is speciating and thus not capable of fully capturing the true genetic structure of

491   these species. The conflicting possibilities presented above and this issue of recent speciation outpacing

492   divergence in the COI marker, highlight the need for a full genomic study of this clade to determine the

493   validity of these species. Detailed ecological observations would also be highly desirable to establish

494   field identification guidelines for each species, if indeed they can be reliably differentiated in the field.

495   **5.   Conclusion**

496   The phylogenetic signal of sociality in *Gobiodon* could not be conclusively resolved. However, we found

497   a combination of life-history and ecological effects best predicted sociality in these species. Previous

498   research suggests that sociality is probably quite plastic in *Gobiodon* and supports the idea of

499   phylogenetic independence of sociality (Hing et al., 2018). Our study revealed a relationship between

500   sociality and the interaction between ecological and life-history factors. This provides good evidence for

501   a link between these correlates and sociality in this genus, which should now be tested experimentally in

502   order to demonstrate causality. We also highlight the need for full genomic studies of *G. spilophthalmus*,

503   *G. acicularis* and *G. ceramensis* which have caused substantial confusion in the literature at the time of

504   writing. With continued advances in genomic sequencing we anticipate this study will encourage future

505   research to resolve the validity of these species. Issues of species identification aside, this study

506   complements the admirable body of research conducted on terrestrial organisms by presenting a novel

507   perspective of ecological and life-history traits which have likely influenced the evolution of sociality.

508   Work on terrestrial organisms has been instrumental in developing theories of social evolution.

509   However, these terrestrially derived theories have only recently been tested against organisms

510   displaying non-conventional life-history strategies.

522   **8.   References**

523   Ackerly, D.D., Donoghue, M.J., 1995. Phylogeny and ecology reconsidered. Journal of Ecology 83, 730-

524   733.

525   Agnarsson, I., 2002. Sharing a web - On the relation of sociality and kleptoparasitism in theridiid spiders

526   (Theridiidae, Araneae). J. Arachnol. 30, 181-188.

527   Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J.L., Kovacic, M., Zardoya, R., Ruber, L., 2013.

528   Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. Mol. Phylogenet.

529   Evol. 69, 619-633.

530   Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for

531   PCR-based techniques. NUCLEIC ACIDS RES. 25, 4692-4693.

532   Armitage, K.B., 1981. Sociality as a life-history tactic of ground squirrels. Oecologia 48, 36-49.

533   Arnold, K.E., Owens, I.P.F., 1998. Cooperative breeding in birds: a comparative test of the life history

534   hypothesis. Proceedings of the Royal Society B-Biological Sciences 265, 739-745.

535   Avilés, L., Harwood, G., 2012. A Quantitative Index of Sociality and Its Application to Group-Living

536   Spiders and Other Social Organisms. Ethology 118, 1219-1229.

537   Avise, J.C., Shapiro, D.Y., 1986. Evaluating kinship of newly settled juveniles within social groups of the

538   coral reef fish Anthias squamipinnis. Evolution 40, 1051-1059.

539   Bekoff, M., Diamond, J., Mitton, J.B., 1981. Life-history patterns and sociality in canids: Body size,

540   reproduction, and behavior. Oecologia 50, 386-390.

541   Blomberg, S.P., Garland Jr, T., Ives, A.R., 2003. Testing for phylogenetic signal in comparative data:

542   Behavioral traits are more labile. Evolution 57, 717-745.

543 Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A.,

544 Drummond, A.J., 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLOS

545 Computational Biology 10, e1003537.

546 Bourke, A.F.G., 2011. Principles of social evolution. Oxford University Press Oxford.

547 Bourke, A.F.G., 2014. Hamilton's rule and the causes of social evolution. Philosophical Transactions of

548 the Royal Society B-Biological Sciences 369, 10.

549 Brandl, S.J., Goatley, C.H.R., Bellwood, D.R., Tornabene, L., 2018. The hidden half: Ecology and evolution

550 of cryptobenthic fishes on coral reefs. Biological Reviews.

551 Briga, M., Pen, I., Wright, J., 2012. Care for kin: within-group relatedness and allomaternal care are

552 positively correlated and conserved throughout the mammalian phylogeny. Biology Letters 8, 533-536.

553 Brooks, K.C., Maia, R., Duffy, J.E., Hultgren, K.M., Rubenstein, D.R., 2017. Ecological generalism facilitates

554 the evolution of sociality in snapping shrimps. Ecology Letters 20, 1516-1525.

555 Brown, J.L., 1974. Alternate Routes to Sociality in Jays—With a Theory for the Evolution of Altruism and

556 Communal Breeding. American Zoologist 14, 63-80.

557 Buston, P., 2003. Forcible eviction and prevention of recruitment in the clown anemonefish. Behavioral

558 Ecology 14, 576-582.

559 Buston, P.M., Bogdanowicz, S.M., Wong, A., Harrison, R.G., 2007. Are clownfish groups composed of

560 close relatives? An analysis of microsatellite DNA variation in *Amphiprion percula*. Mol. Ecol. 16, 3671-

561 3678.

562 Buston, P.M., Fauvelot, C., Wong, M.Y.L., Planes, S., 2009. Genetic relatedness in groups of the humbug

563 damselfish *Dascyllus aruanus*: small, similar-sized individuals may be close kin. Mol. Ecol. 18, 4707-4715.

564 Buston, P.M., Wong, M.Y.L., 2014. Why Some Animals Forgo Reproduction in Complex Societies

565 Behaviors of coral reef fishes provide strong support for some major new ideas about the evolution of

566 cooperation. Am. Scientist 102, 290-297.

567   Chapple, D.G., 2003. Ecology, life-history, and behavior in the Australian Scincid genus *Egernia,* with

568   comments on the evolution of complex sociality in lizards. Herpetol. Monogr. 17, 145-180.

569   Cole, K.S., 2011. Patterns of reproductive morphology in the genus Gobiodon (Teleostei: Gobiidae).

570   Environmental Biology of Fishes 92, 323-335.

571   Cole, K.S., Hoese, D.F., 2001. Gonad morphology, colony demography and evidence for hermaphroditism

572   in Gobiodon okinawae (Teleostei, Gobiidae). Environmental Biology of Fishes 61, 161-173.

573   Dey, C.J., O'Connor, C.M., Wilkinson, H., Shultz, S., Balshine, S., Fitzpatrick, J.L., 2017. Direct benefits and

574   evolutionary transitions to complex societies. Nature ecology & evolution 1, 0137.

575   Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian Phylogenetics with BEAUti and the

576   BEAST 1.7. Molecular Biology and Evolution 29, 1969-1973.

577   Duchene, D., Klanten, S.O., Munday, P.L., Herler, J., van Herwerden, L., 2013. Phylogenetic evidence for

578   recent diversification of obligate coral-dwelling gobies compared with their host corals. Mol.

579   Phylogenet. Evol. 69, 123-132.

580   Edwards, S.V., Naeem, S., 1993. The Phylogenetic Component of Cooperative Breeding in Perching Birds.

581   American Naturalist 141, 754-789.

582   Emlen, S.T., 1982. The evolution of helping 1. An ecological constraints model. American Naturalist 119,

583   29-39.

584   Faulkes, C.G., Bennett, N.C., Bruford, M.W., Obrien, H.P., Aguilar, G.H., Jarvis, J.U.M., 1997. Ecological

585   constraints drive social evolution in the African mole-rats. Proceedings of the Royal Society B-Biological

586   Sciences 264, 1619-1627.

587   Fowler, H.W., 1944. Fishes obtained in the New Hebrides by Dr. Edward L. Jackson. Proceedings of the

588   Academy of Natural Sciences of Philadelphia 96, 155-371.

589   Fox, J., Weisberg, S., 2011. An {R} Companion to Applied Regression. Sage Publications, Thousand Oaks

590   CA.

591   Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for

592   Windows 95/98/NT. Nucleic acids symposium series. [London]: Information Retrieval Ltd., c1979-c2000.,

593   pp. 95-98.

594   Halliwell, B., Uller, T., Holland, B.R., While, G.M., 2017. Live bearing promotes the evolution of sociality

595   in reptiles. Nature communications 8, 2030.

596   Hamilton, W.D., 1964. The genetical evolution of social behaviour. II. Journal of Theoretical Biology 7,

597   17-52.

598   Harold, A.S., Winterbottom, R., Munday, P.L., Chapman, R.W., 2008. Phylogenetic relationships of Indo-

599   Pacific coral gobies of the genus Gobiodon (Teleostei : Gobiidae), based on morphological and molecular

600   data. Bull. Mar. Sci. 82, 119-136.

601   Harvey, P.H., Pagel, M., Read, A., Nee, S., 1995. Further remarks on the role of phylogeny in comparative

602   ecology. Naturalist 140, 421-446.

603   Hatchwell, B.J., Komdeur, J., 2000. Ecological constraints, life history traits and the evolution of

604   cooperative breeding. Animal Behaviour 59, 1079-1086.

605   Helfman, G.S., Winkelman, D.L., 1997. Threat sensitivity in bicolor damselfish: Effects of sociality and

606   body size. Ethology 103, 369-383.

607   Herler, J., Bogorodsky, S.V., Suzuki, T., 2013. Four new species of coral gobies (Teleostei: Gobiidae:

608   Gobiodon), with comments on their relationships within the genus. Zootaxa 3709, 301-329.

609   Herler, J., Koblmuller, S., Sturmbauer, C., 2009. Phylogenetic relationships of coral-associated gobies

610   (Teleostei, Gobiidae) from the Red Sea based on mitochondrial DNA data. Marine Biology 156, 725-739.

611   Hing, M.L., Klanten, O.S., Dowton, M., Brown, K.R., Wong, M.Y.L., 2018. Repeated cyclone events reveal

612   potential causes of sociality in coral-dwelling Gobiodon fishes. PLoS One 13, e0202407.

613   Hing, M.L., Klanten, O.S., Dowton, M., Wong, M.Y.L., 2017. The Right Tools for the Job: Cooperative

614   Breeding Theory and an Evaluation of the Methodological Approaches to Understanding the Evolution

615   and Maintenance of Sociality. Frontiers in Ecology and Evolution 5.

616   Hobbs, J.P.A., Munday, P.L., 2004. Intraspecific competition controls spatial distribution and social

617   organisation of the coral-dwelling goby Gobiodon histrio. Marine Ecology Progress Series 278, 253-259.

618   Hobbs, J.P.A., Munday, P.L., Jones, G.P., 2004. Social induction of maturation and sex determination in a

619   coral reef fish. Proceedings of the Royal Society B-Biological Sciences 271, 2109-2114.

620   Holcroft, N.I., 2005. A molecular analysis of the interrelationships of tetraodontiform fishes

621   (Acanthomorpha: Tetraodontiformes). Mol. Phylogenet. Evol. 34, 525-544.

622   Hughes, W.O.H., Oldroyd, B.P., Beekman, M., Ratnieks, F.L.W., 2008. Ancestral monogamy shows kin

623   selection is key to the evolution of eusociality. Science 320, 1213-1216.

624   Jennions, M.D., Macdonald, D.W., 1994. Cooperative Breeding in Mammals. Trends in Ecology &

625   Evolution 9, 89-93.

626   Jetz, W., Rubenstein, D.R., 2011. Environmental Uncertainty and the Global Biogeography of

627   Cooperative Breeding in Birds (vol 21, pg 72, 2011). Current Biology 21, 438-438.

628   Kokko, H., Johnstone, R.A., Wright, J., 2002. The evolution of parental and alloparental effort in

629   cooperatively breeding groups: When should helpers pay to stay? Behavioral Ecology 13, 291-300.

630   Kokko, H., Ekman, J., 2002. Delayed dispersal as a route to breeding: Territorial inheritance, safe havens,

631   and ecological constraints. American Naturalist 160, 468-484.

632   Kruckenhauser, L., Pinsker, W., Haring, E., Arnold, W., 1999. Marmot phylogeny revisited: Molecular

633   evidence for a diphyletic origin of sociality. J. Zool. Syst. Evol. Res. 37, 49-56.

634   Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0

635   for Bigger Datasets. Molecular biology and evolution 33, 1870-1874.

636   Kuwamura, T., Yogo, Y., Nakashima, Y., 1994. Population dynamics of goby Paragobiodon

637   echinocephalus and host coral Stylophora pistillata. Marine Ecology Progress Series 103, 17-23.

638   Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: Combined Selection of Partitioning

639   Schemes and Substitution Models for Phylogenetic Analyses. Molecular Biology and Evolution 29, 1695-

640   1701.

641   Li, C., Ortí, G., Zhang, G., Lu, G., 2007. A practical approach to phylogenomics: the phylogeny of ray-

642   finned fish (Actinopterygii) as a case study. BMC Evolutionary Biology 7, 44.

643   Lukas, D., Clutton-Brock, T., 2012. Cooperative breeding and monogamy in mammalian societies.

644   Proceedings of the Royal Society B: Biological Sciences 279, 2151-2156.

645   Michael, D.R., Cunningham, R.B., Lindenmayer, D.B., 2010. The social elite: Habitat heterogeneity,

646   complexity and quality in granite inselbergs influence patterns of aggregation in Egernia striolata

647   (Lygosominae: Scincidae). Austral Ecol. 35, 862-870.

648   Munday, P., Harold, A., Winterbottom, R., 1999. Guide to coral-dwelling gobies, genus Gobiodon

649   (Gobiidae), from Papua New Guinea and the Great Barrier Reef. Revue française d'aquariologie 26, 53-

650   58.

651   Munday, P.L., Caley, M.J., Jones, G.P., 1998. Bi-directional sex change in a coral-dwelling goby.

652   Behavioral Ecology and Sociobiology 43, 371-377.

653   Munday, P.L., Cardoni, A.M., Syms, C., 2006. Cooperative growth regulation in coral-dwelling fishes.

654   Biology Letters 2, 355-358.

655   Munday, P.L., Jones, G.P., 1998. The ecological implications of small body size among coral-reef fishes.

656   Oceanography and Marine Biology 36, 373-411.

657   Munday, P.L., Jones, G.P., Caley, M.J., 1997. Habitat specialisation and the distribution and abundance of

658   coral-dwelling gobies. Marine Ecology Progress Series 152, 227-239.

659   Munday, P.L., Wilson, S.K., 1997. Comparative efficacy of clove oil and other chemicals in

660   anaesthetization of Pomacentrus amboinensis, a coral reef fish. Journal of Fish Biology 51, 931-938.

661   Nakashima, Y., Kuwamura, T., Yogo, Y., 1996. Both-ways sex change in monogamous coral gobies,

662   *Gobiodon spp*. Environmental Biology of Fishes 46, 281-288.

663   Nowicki, J.P., O'Connell, L.A., Cowman, P.F., Walker, S.P.W., Coker, D.J., Pratchett, M.S., 2018. Variation

664   in social systems within Chaetodon butterflyfishes, with special reference to pair bonding. PLoS One 13,

665   e0194465.

666   Pagel, M., 1999. Inferring the historical patterns of biological evolution. Nature 401, 877-884.

667   Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language.

668   Bioinformatics 20, 289-290.

669   Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D.,R Core Team, 2018. nlme: Linear and nonlinear mixed

670   effects models. R package version 3.1-137. Available at h ttp://CRAN. R-project. org/package= nlme.

671   Puillandre, N., Lambert, A., Brouillet, S., Achaz, G., 2012. ABGD, Automatic Barcode Gap Discovery for

672   primary species delimitation. Mol. Ecol. 21, 1864-1877.

673   Rambaut, A., Drummond, A., Xie, D., Baele, G., Suchard, M.A., 2018. Tracer v1.7.

674   http://tree.bio.ed.ac.uk/software/tracer/.

675   Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things).

676   Methods in Ecology and Evolution 3, 217-223.

677   Riehl, C., 2011. Living with strangers: direct benefits favour non-kin cooperation in a communally nesting

678   bird. Proceedings of the Royal Society B-Biological Sciences 278, 1728-1735.

679   Riehl, C., 2013. Evolutionary routes to non-kin cooperative breeding in birds. Proceedings of the Royal

680   Society B-Biological Sciences 280, 7.

681   Rowley, I., Russell, E., 1990. Splendid fairy-wrens: demonstrating the importance of longevity. In: Stacey,

682   P.K., WD (Ed.), Cooperative breeding in birds: long-term studies of ecology and behavior. Cambridge

683   University Press, Cambridge, pp. 3-30.

684   Rubenstein, D.R., Abbot, P., 2017. Comparative social evolution. Cambridge University Press.

685   Rubenstein, D.R., Lovette, I.J., 2007. Temporal environmental variability drives the evolution of

686   cooperative breeding in birds. Current Biology 17, 1414-1419.

687   Schneider, T.C., Kappeler, P.M., 2014. Social systems and life-history characteristics of mongooses.

688   Biological Reviews 89, 173-198.

689   Schürch, R., Accleton, C., Field, J., 2016. Consequences of a warming climate for social organisation in

690   sweat bees. Behavioral Ecology and Sociobiology 70, 1131-1139.

691   Shibukawa, K., Suzuki, T., Aizawa, M., 2013. Gobiodon aoyagii, a new coral goby (Actinopterygii,

692   Gobiidae, Gobiinae) from the west Pacific, with redescription of a similarly-colored congener Gobiodon

693   erythrospilus Bleeker, 1875. Bulletin of the National Museum of Nature and Science, Series A 39, 143-

694   165.

695   Shultz, S., Opie, C., Atkinson, Q.D., 2011. Stepwise evolution of stable sociality in primates. Nature 479,

696   219-U296.

697   Smorkatcheva, A.V., Lukhtanov, V.A., 2014. Evolutionary association between subterranean lifestyle and

698   female sociality in rodents. Mamm. Biol. 79, 101-109.

699   Stacey, P.B., Ligon, J.D., 1991. The benefits-of-philopatry hypothesis for the evolution of cooperative

700   breeding - variation in territory quality and group-size effects. American Naturalist 137, 831-846.

701   Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large

702   phylogenies. Bioinformatics 30, 1312-1313.

703   Steinke, D., de Waard, J.R., Gomon, M.F., Johnson, J.W., Larson, H.K., Lucanus, O., Moore, G.I., Reader,

704   S., Ward, R.D., 2017. DNA barcoding the fishes of Lizard island (Great Barrier Reef). Biodivers. Data J. 5.

705   Thacker, C.E., Roje, D.M., 2011. Phylogeny of Gobiidae and identification of gobiid lineages. Systematics

706   and Biodiversity 9, 329-347.

707   Thompson, V.J., Munday, P.L., Jones, G.P., 2007. Habitat patch size and mating system as determinants

708   of social group size in coral-dwelling fishes. Coral Reefs 26, 165-174.

709   Untersteggaber, L., Mitteroecker, P., Herler, J., 2014. Coral architecture affects the habitat choice and

710   form of associated gobiid fishes. Marine Biology 161, 521-530.

711   Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcoding Australia's fish

712   species. Philosophical Transactions of the Royal Society B: Biological Sciences 360, 1847-1857.

713   Westoby, M., Leishman, M.R., Lord, J.M., 1995. On misinterpreting the 'phylogenetic correction'. Journal

714   of Ecology 83, 531-534.

715   Westoby, M., Leishman, M., Lord, J., 1995. Further remarks on phylogenetic correction. Journal of

716   Ecology 83, 727-729.

717   While, G.M., Uller, T., Wapstra, E., 2009. Family conflict and the evolution of sociality in reptiles.

718   Behavioral Ecology 20, 245-250.

719   Wong, M.Y.L., 2010. Ecological constraints and benefits of philopatry promote group-living in a social

720   but non-cooperatively breeding fish. Proceedings of the Royal Society B-Biological Sciences 277, 353-

721   358.

722   Wong, M.Y.L., 2011. Group Size in Animal Societies: The Potential Role of Social and Ecological

723   Limitations in the Group-Living Fish, *Paragobiodon xanthosomus*. Ethology 117, 638-644.

724   Wong, M.Y.L., Buston, P.M., 2013. Social Systems in Habitat-Specialist Reef Fishes: Key Concepts in

725   Evolutionary Ecology. Bioscience 63, 453-463.

726   Wong, M.Y.L., Buston, P.M., Munday, P.L., Jones, G.P., 2007. The threat of punishment enforces

727   peaceful cooperation and stabilizes queues in a coral-reef fish. Proceedings of the Royal Society B-

728   Biological Sciences 274, 1093-1099.