The rapid divergence of the Antarctic crinoid species *Promachocrinus kerguelensis*

Running title: Phylogeography of Antarctic crinoids

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Abstract

Climatic oscillations in Antarctica caused a succession of expansion and reduction of the ice sheets covering the continental shelf of the Southern Ocean. For marine invertebrates, these successions are suspected to have driven allopatric speciation, endemism and the prevalence of cryptic species, leading to the so-called Antarctic 'biodiversity pump' hypothesis. Here we took advantage of the recent sampling effort influenced by the International Polar Year (2007-8) to test for the validity of this hypothesis for 1,797 samples of two recognized crinoid species: *Promachocrinus kerguelensis* and *Florometra mawsoni*. Species delimitation analysis identified seven phylogroups. As previously suggested, *Promachocrinus kerguelensis* forms a complex of six cryptic species. Conversely, despite the morphological differences, our results show that *Florometra mawsoni* is a lineage nested within *Promachocrinus kerguelensis*. It suggests that *Florometra mawsoni* and *Promachocrinus kerguelensis* belong to the same complex of species. Furthermore, this study indicates that over time and space the different sectors of the Southern Ocean show a remarkable rapid turn-over in term of phylogroups composition and also of genetic variants within phylogroups. We argue that strong "apparent" genetic drift causes this rapid genetic turn-over. Finally, we dated the last common ancestor of all phylogroups at less than 1,000 years, raising doubts on the relevance of the Antarctic "biodiversity pump" for this complex of species. This work is a first step towards a better understanding of how life is diversifying in the Southern Ocean.

Introduction

During the last decade following the last International Polar Year (2007-8) a huge effort has been made to better understand Antarctic marine ecosystems and one of the major outcomes has been the creation of a reference barcode database (BOLD) that reconciles morphologically recognized species with a unique COI fragment. This opened to a new era of discoveries that allowed reassess some of the main known characteristics of marine Antarctic ecosystems, including eurybathy, excess in brooding species, high rate of endemism, rapid diversification and cryptic speciation as a consequence of the biodiversity pump (Clarke & Crame, 1989). Recent biodiversity assessments using molecular tools have revealed an increasing number of cryptic species in teleost fishes as well as Echinodermata, Mollusca, Arthropoda, or Annelida (see (Cornils & Held, 2014; Griffiths, 2010; Rogers, 2007) for comprehensive reviews). Cryptic species have been shown to be homogeneously distributed among taxa and biogeographic regions (Pfenninger & Schwenk, 2007) and Antarctica may well be no exception.

The Southern Ocean is known to have undergone several glaciation events (Clarke & Crame, 1989; Clarke, Crame, Stromberg, & Barker, 1992; Thatje, Hillenbrand, Mackensen, & Larter, 2008). Massive ice sheet advance and retreat seem to have bulldozed the entire continental shelf several times during the last 25 Mya. These events are thought to be driven by Milankovitch cycles. These cycles may be among the strongest evolutionary forces that have shaped Antarctic terrestrial and marine biodiversity (Clarke et al., 1992; Thatje et al., 2008; Thatje, Hillenbrand, & Larter, 2005). Thatje *et al.* (2005; 2008) hypothesized that vicariant speciation could have occurred during the glacial periods on the Antarctic continental shelf, within multiple ice-free refugia like polynya. As lineages evolved independently during the glacial period, they accumulated genetic differences that lead to reproductive isolation and probably to the formation of cryptic species. During interglacial periods, barriers to gene flow may have been removed allowing for secondary contact between the vicarious lineages (Heimeier, Lavery, & Sewell, 2010; Thatje et al., 2005; 2008; Thornhill, Mahon, Norenburg, & Halanych, 2008). An alternative hypothesis is based on the idea that Antarctic continental shelf may be understood as a species flock generator (Eastman & McCune, 2000; Lecointre et al., 2013).

Here, we have analyzed COI sequences of 1,797 individuals of two crinoid species:

85 *Promachocrinus kerguelensis* (Carpenter, 1888) and *Florometra mawsoni* (AH Clark, 1937),
86 endemic to the Southern Ocean. These species represent the most abundant crinoid species in
87 the Southern Ocean (Eléaume, Hemery, Roux, & Améziane, 2014; Speel & Dearborn, 1983).
88 They are morphologically distinct (but see (Eléaume, 2006) for counter arguments) and
89 genetically close (Hemery, Améziane, & Eléaume, 2013a). These species are thought to have
90 a reproduction cycle that involves external fertilization that could result in a large dispersal
91 potential. *P. kerguelensis* produces positively buoyant ovocytes that, after fertilization, may
92 remain in the plankton for weeks or even months (McClintock & Pearse, 1987). Some adults
93 of *P. kerguelensis* have also been observed swimming (Eléaume, unpublished observations),
94 and some adults of *F. mawsoni*, though never observed swimming in situ, have shown this
95 ability in tanks (Eléaume, unpublished observations).

96 During the last decade, numerous specimens of crinoids have been collected from all around
97 Antarctica and sub-Antarctic islands. Over 3,000 specimens attributed to 45 species have been
98 sampled (Eléaume et al., 2014). Species such as *Promachocrinus kerguelensis* and
99 *Florometra mawsoni* are represented by a large number of well distributed specimens.
100 Knowlton (1993; 2000) predicted for Southern Ocean organisms that an increase in sampling
101 effort and the application of genetic tools, would reveal cryptic species. The analysis of
102 *P. kerguelensis* COI barcode fragment suggested that this species may constitute such an
103 example of a complex of unrecognized species. Wilson *et al.* (2007) identified six lineages in
104 the Atlantic sector whereas Hemery *et al.* (2012) extending the analysis of Wilson to the
105 entire Southern Ocean, identified seven lineages. As no obvious morphological character have
106 been shown to distinguish between these lineages (Eléaume, 2006), they may represent true
107 cryptic species. However, Hemery *et al.* (2012) using nuclear markers have shown that the six
108 identified COI lineages may only represent three distinct entities. All of the six or seven
109 lineages are circumpolar in distribution, sympatric and eurybathic but show various levels of
110 connectivity that depend on the lineage and the geographical area.

111 Here we analyze the pooled COI datasets of *P. kerguelensis* and *F. mawsoni* of Wilson *et al.*
112 (2007) and Hemery *et al.* (2012) using different approaches to separate different sources of
113 genetic variation, i.e. differences due to diversification (clade), time (year collected), space
114 (geographical origin of samples). We first analyze the phylogenetic relationships within
115 *P. kerguelensis* sensu largo using different species delineation methods. We then measure the
116 genetic turn-over, within each phylogroup sampled at a given location. Using a population
117 genetics coalescent framework, we estimated an extraordinary small effective population size

that corresponds to a mutation rate that is larger than previously reported before. We then discuss our results in the light of the climate history of the Southern Ocean.

Materials and methods

Sequences

We included 13 sequences of *P. kerguelensis* from Wilson *et al.* (2007), for which we had precise information about the place and the date of sampling. We have also included 1303 sequences of *P. kerguelensis* from Hemery *et al.* (2012) together with 479 sequences of *F. mawsoni* (Hemery *et al.,* in preparation). A total of 1,797 COI sequences were used for this study. All specimens were collected between 1996 and 2010 in seven geographical regions that were described in Hemery *et al.* (Hemery et al., 2012): Kerguelen Plateau (KP), Davis Sea (DS), Terre Adélie (TA), Ross Sea (RS), Amundsen Sea (AS), West Antarctic Peninsula (WAP), East Weddell Sea (EWS). We however further split the Scotia Arc into Scotia Arc East (SAE) and Scotia Arc West (SAW) as there can be as much as 2,000 km between them. The counts of all sequences of all locations are reported in Table S1.

DNA extraction, PCR and sequencing

For this analysis, no additional sequences have been produced. For DNA extraction and PCR procedures see (Hemery et al., 2012; Wilson et al., 2007) and 2013. A total of 1797 554-bp sequences of the barcode region of cytochrome c oxidase subunit I (COI) were amplified using the Folmer *et al.* (1994) primers and other specific primers described in Hemery *et al*. (2012). All COI sequences from Hemery *et al.* (2012) have been made easily available through a data paper article (Hemery et al., 2013a).

Species delimitation

We used three independent species delimitation methods: Generalized Mixed Yule Coalescent (GYMC, (Pons et al., 2006)), Automatic Barcode Gap Discovery (ABGD, (Puillandre, Lambert, Brouillet, & Achaz, 2011)) and Poisson Tree Process (PTP, (Zhang, Kapli, Pavlidis, & Stamatakis, 2013)) to estimate the number of phylogroups in our sample. For all these methods, we used all the 199 unique haplotypes of the dataset to have legible results and a faster computation time. The phylogenetic tree used for PTP was constructed by Neighbor Joining method the BioNJ implementation (Gascuel, 1997) in *Seaview* software (Gouy, Guindon, & Gascuel, 2010) version 3.2. PTP analysis was conducted online (http://species.h-its.org/ptp/). The ultrametric tree needed for GYMC method was constructed using *BEAST* software (Bayesian Evolutionary Analysis Sampling Trees, (Drummond, Suchard, Xie, &

151 Rambaut, 2012) version 1.7). GYMC analysis was conducted online (http://species.h-
152 its.org/gmyc/). ABGD analysis was conducted online
153 (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html).

154 ***Assessing the temporal structure within phylogroups***

155 We characterized the temporal structures of our samples in pairwise comparisons using both
156 fixation index F_{ST} as well as a non-parametric structure test based on K^*s by Hudson *et al.*
157 (1992). All p-values were estimated using permutations as described in Hudson *et al.* (1992).
158 A web-interface for the latter can be found at http://wwwabi.snv.jussieu.fr/public/mpweb/.

159 ***Estimation of the mutation rate and the population effective size***

160 As described by Fu (2001) one can use temporal data to estimate the mutation rate (μ). In
161 summary, the method assumes that the population is sampled in at least two time points (say
162 T_1 and then later T_2) with multiple sequences in T_1. The expected average pairwise differences
163 between sequences taken from the two time points (K_{12}) equals the average pairwise
164 differences of sequences within the past time point (K_{11}) plus the difference accumulated
165 since then. Mathematically, it is expressed as $E[K_{12}] = E[K_{11}] + (T_2-T_1)\mu$. Because the time
166 difference between the samples (T_2-T_1) is known, the mutation rate can be estimated from the
167 observed pairwise differences: $\mu = (K_{12}-K_{11})/(T_2-T_1)$. This idea can be expanded when more
168 data are available in other time points (Fu, 2001).

169 Once μ is known, the average pairwise difference can be used to estimate the effective
170 population size (*Ne*). Under the standard neutral model, the pairwise difference within groups
171 of the same sample equals $2Ne\mu$, when μ is expressed as a rate by generations. Here, we
172 assumed one generation every three year to estimate *Ne* (Clark, 1921).

173 ***Estimation of the time of divergence between phylogroups***

174 For visualization purposes, an ultrametric tree was constructed with the unique sequences by
175 UPMGA (Michener & Sokal, 1957) as implemented in the *Phylip* package (Felsenstein, 1989)
176 version 3.6. Using the estimated mutation rate, we derived a time of divergence within and
177 between phylogroups from the average sequence differences.

178

179 ***Polymorphisms analysis***

180 We used *Dnasp* software version 5.10 (Librado & Rozas, 2009) and the program used to
181 study genetic structure to study the SFS (Site Frequency Spectrum) within phylogroups as
182 well as two of its summary statistics: nucleotide diversity (π) and number of polymorphic
183 sites (S). We then tested neutrality using Tajima's D (Tajima, 1989), Fu and Li's F* and D*
184 (Fu & Li, 1993), Achaz's Y* (Achaz, 2008), that is immune to sequencing errors, and the
185 Strobeck (Strobeck, 1987) haplotype test.

186 ***Assessment of migration effect on Ne estimation through simulations***

187 To assess the potential effect of migrants from a distant population on the estimation of μ and
188 *Ne*, we used coalescent simulations where two samples of 20 sequences each are taken 3N
189 generations apart in a focal population of size *N*. We assume a large unsampled ghost
190 population of size *10N* that exchange migrants with the focal one at rate $M=4Nm$, where *m* is
191 the migration rate per generation. For values of *M* ranging from 0.001 (very low) to 1000
192 (very large), we applied the Fu (Fu, 2001) method to estimate *Ne*, with 10^3 replicates for each
193 *M* value. The program is based on a C++ library developed by G. Achaz that is available upon
194 request.

Results

P. kerguelensis and F. mawsoni are composed of seven phylogroups

We first reinvestigated the species delimitation of the sample using three independent approaches (GMYC, ABGD and PTP) that cluster the unique sequences by phylogroup. We adopt in this manuscript a nominalist definition of species. However, *F. mawsoni*, a nominal species, is nested within *P. kerguelensis*, another nominal species, lineages. To avoid confusion, we have decided to use the term "phylogroup" in the rest of the manuscript to designate any well-supported cluster of sequences derived from species delineation approaches.

Two of the three methods estimate a similar number of phylogroups: PTP estimates that there are nine phylogroups in the sample whereas ABGD reports 5-7 phylogroups, depending on the chosen prior value. Looking at the proposed partitions, we have chosen to consider seven different phylogroups for this study that are reported as phylogroups A-G in Fig. 1. Interestingly, these phylogroups correspond to the A-F groups proposed by Wilson *et al.* (2007), plus a 'new' group (G) that corresponds to the *F. mawsoni* species. The two extra sub-splits suggested by the PTP method are indicated as C1/C2 and E1/E2 on Fig. 1. Note that PTP only seeks monophyletic groups and thus must split the C group into the C1/C2 subgroups.

In contrast with the two previous methods, depending on the sequence evolution model we used and the *BEAST* parameters, GMYC reports between 3 and 51 phylogroups with non-overlapping grouping between individuals. As GMYC is very sensitive in our case to the chosen parameters, it is here unreliable and we chose to not consider the GMYC partitions.

Genetic distance between phylogroups are similar to the distance of each phylogroup to the G group. However, G group corresponds to a nominal species (*F. mawsoni)* which suggests that the A-G phylogroups could correspond to seven different yet undescribed species.

A very rapid local turn-over

Relative abundance of phylogroups: Two of the analyzed locations (Ross Sea and East Weddell Sea) were densely sampled (at least 50 sequences) at two (or more) different time points. We therefore took advantage of these time series to evaluate the turnover of the resident crinoids from year to year. The analysis of the relative abundance of the various

225 phylogroups shows that for both locations the genetic composition is highly unstable through
226 time (Fig. 2a and 2c). Phylogroup composition in EWS is stable from 1996 to 2004, and
227 changes drastically in 2005 when phylogroup C has not been sampled. This pattern leads to a
228 highly significant difference between all years (Fisher exact test, $P = 4.7\ 10^{-10}$). Similarly, the
229 composition of the RS location is also highly significantly different between 2004 and 2008
230 (Fisher exact test, $P = 3.2\ 10^{-11}$); in this case, phylogroup A is replaced by phylogroups B and
231 E.

232 Other locations display a single sampling event (AS, DS, TA) or reduced number of
233 sequences over several sampling events (e.g. SAW: 2 sequences in 2000, 11 in 2002, 7 in
234 2006 and 68 in 2009), and cannot be used to robustly assess the stability of phylogroup
235 relative abundance.

236 **Turn-over within the phylogroups:** We then measured the replacement of genetic variants
237 within the phylogroups. To do so, we considered samples with at least 10 sequences of the
238 same phylogroup in the same location at two different time points. The pairwise comparisons
239 for such groups are all reported in Table 1 and we provide two graphical representations for
240 phylogroup D in the RS location (Fig. 2b) and phylogroup C in the EWS location (Fig. 2d). In
241 two cases (phylogroup C in EWS and phylogroup D in RS), we found a significant difference
242 in the genetic composition within each phylogroups even when they are separated by only few
243 years, again suggesting an unexpectedly high turn-over of the genetic pool of these crinoids.
244 Interestingly, the temporal differentiation of phylogroup E within EWS is already F_{ST}=4% in
245 four years, that is not significant because of the small sample size (10 and 11 respectively).
246 Only the two samples of phylogroup G in EWS, that are separated by a single year, show no
247 sign of temporal differentiation (F_{ST}=1%).

248 ***Estimation of the mutation rate and the effective population size***

249 Using the robust yet elegant approach devised by Fu (2001), we estimated the yearly mutation
250 rates (μ) from the same sample comparisons (i.e. phylogroups with two or more samples of 10
251 or more sequences within the same location). Furthermore, we also estimated the
252 corresponding effective population size (Ne) assuming a generation time of three years.
253 Results (Table 2) shows that all estimated mutation rates are 10^{-5}-10^{-4} /bp/year, with a mean
254 of 8.10^{-5} /bp/year. Results also show that the effective sizes are very low, on the order of 2-7
255 (Table 2). The very low effective size corresponds to the unexpected high turn-over of the
256 genetic pool that we observe within the phylogroup at the same location.

257 ***Polymorphism analysis***

258 For samples of 10 sequences or more (with the same year and same location), we have
259 analyzed the characteristics of segregating polymorphisms using four neutrality tests:
260 Tajima's D (Tajima, 1989), Fu and Li's F* and D* (Fu & Li, 1993), Achaz's Y* (Achaz,
261 2008) and Strobeck P-values (Strobeck, 1987). Results (Fig. 3) show almost no deviation
262 from the standard neutral model, except for the Fu and Li's F* and D*. This statistics points
263 to a clear excess of singletons. However, singletons likely contain sequencing errors that
264 strongly skew these statistics (Achaz, 2008). The Y statistics that ignores singletons exhibits a
265 distribution that is very close to the one expected under the standard neutral model. We
266 therefore conclude that the observed polymorphisms suggest that basic assumptions of the
267 standard neutral model (i.e. constant population size, no migration, no structure and no
268 selection) cannot be rejected for these crinoid phylogroups.

269 ***Assessing the effect of migration using simulations***

270 To test whether migration could accelerate the genetic turn-over of a species on a given
271 location, we studied a simple model where the sampled location exchanges migrants with a
272 large pool of panmictic individuals. One expects that a constant arrival of migrants from this
273 large pool could interfere with the estimation of effective size. We thus simulated a model
274 where the sampled population has a size *N* and the pool 10*N*. We studied the impact of the
275 migration rate on the estimation of Ne using the same method than above (2001). Results (Fig.
276 4) show that at low migration rate, the estimator is unaffected whereas it is inflated up to 11*N*
277 at high migration rate. This shows that the existence of a large pool from where migrants can
278 come into the sampled population can only inflate the estimation of *Ne* and therefore cannot
279 explain the low *Ne* we have estimated.

280 ***Phylogroups divergence***

281 We then computed an estimate for the times of split between the seven phylogroups using our
282 population genetics estimation of the mutation rate (8.10^{-5} mutations /bp /year). Our estimates
283 are reported in an ultrametric tree (Fig. 5) where the oldest split between the phylogroups is
284 estimated around 400 years. This very short time scale is in sharp contrast with the millions of
285 years of previous estimations based on other estimated mutation rates (3.10^{-8} mutations /bp
286 /year) that were based on echinoderms thought to be separated by the formation of the
287 panama isthmus (Lessios, Kessing, Robertson, & Paulay, 1999).

Discussion

Speciation in Antarctica: the classical view

Due to its extreme environmental conditions, it has been hypothesized that climate driven evolution in Southern Ocean has been more important than in other ecosystems where ecological interactions play a larger role (Rogers, 2012). The diversity and the distribution of many marine taxa in Antarctica has been strongly shaped by climate variation and continental drift leading to dispersal, vicariance and extinction since the break-up of Gondwana (Williams, Reid, & Littlewood, 2003). Indeed, the global climate change at the end of the Eocene related to the continental drift is characteristic of the transition from a temperate climate to the current polar climate in Antarctica (Clarke et al., 1992; Clarke & Crame, 1989). The physical separation of Antarctica, South America and Australia resulted in the cooling of the Southern Ocean, which in turn initiated the physical and climate isolation of this region of the world (Tripati, Backman, Elderfield, & Ferretti, 2005). This isolation was followed by an extensive continental glaciations and the initiation of the Antarctic Circumpolar Current in the Miocene (Clarke et al., 1992; Clarke & Crame, 1989; Thatje et al., 2008). These two processes exacerbated the environmental and biological isolation of the Southern Ocean and are classically recognized as the main explanation to the high level of endemism observed in Antarctica (Clarke & Crame, 1989; Griffiths, Barnes, & Linse, 2009).

The Quaternary was marked by the oscillation of glaciation and interglacial periods, which strongly affected the ice cover present in Antarctica (Davies, Hambrey, Smellie, Carrivick, & Glasser, 2012). These cycles led to severe temperature oscillations and to the succession of large extensions of the ice sheets on the continental shelf followed by retreats of the ice sheets. They have been one of the main drivers of the diversification of species in Antarctica. Indeed, the isolation of populations in ice free refugia during the glacial era, such as polynya or in the deep sea, has been suggested as a mechanism of fragmentation of populations leading to allopatric speciation in the Southern Ocean. This process is called the Antarctic 'biodiversity pump' (Clarke et al., 1992; Clarke & Crame, 1989). The Antarctic 'biodiversity pump' has been proposed to be the main mechanism driving speciation and diversification in Antarctica.

Our knowledge on evolutionary history of Antarctic fauna was classically derived from studies of the systematics and distribution of marine animals relying heavily on the morphology of present and past (fossil) organisms. Such approaches are limited for two main

reasons. First, species morphology may not reflect the true evolutionary relationships between taxa (DeBiasse & Hellberg, 2015). Second, several periods of time lack a reliable fossil record. Today, the emergence of molecular approaches provides a powerful tool to circumvent these limitations. To this regard, the increasing number of phylogeographic studies allow to get a better understanding of the effects of past changes on the current diversity and spatial distribution of organisms (Allcock & Strugnell, 2012). They also allow to accurately assess the distribution of genetic diversity within and among species. Such information is extremely valuable to evaluate and mitigate the impacts of human-induced activities on the biodiversity of the Southern Ocean (Chown et al., 2015). This study take place in this context and by expanding the results of two previous studies (Hemery et al., 2012; Wilson et al., 2007), we aimed to determine the drivers of the diversity of two crinoid taxa in the Southern Ocean, *P. kerguelensis* and *F. mawsoni*.

The concept of species: is Promachocrinus kerguelensis a complex of multiple cryptic species?

The analysis of 1797 sequences using recent species delimitation approaches allowed to identify seven operational taxonomic units. We confirmed the presence of six phylogroups (A-F) identified in two previous study (Hemery et al., 2012; Wilson et al., 2007), but only the PTP method supports the further split between E1 and E2 suggested by Hemery *et al.* (2012). Furthermore, despite their strong morphological differences and monophyly (Fig. 1), our results strongly suggest that *F. mawsoni* (phylogroup G) belongs to the *P. kerguelensis* complex. Indeed, the reciprocal monophyly of G and F and the proximity of E to G/F compare to A-D confirm the affiliation of G as a member of *P. kerguelensis* complex (Fig. 1). Therefore, we corroborated Hemery *et al.* (2012) results showing that *P. kerguelensis* consists in a complex of several cryptic species. We also confirmed Eléaume (2006) conclusion that despite striking exterior morphological differences phylogroup G is also a member of this complex of species, and Hemery et al. (2013b) results based on a multi-markers phylogenetic reconstruction.

Morphological similarities between cryptic species often reflect a recent speciation event (Bickford et al., 2007). In the marine realm, speciation is less associated to morphology than to other phenotypic aspects such as chemical recognition systems (Palumbi, 1994). Knowlton (1993) argued that marine habitats are likely to be filled with cryptic species although rarely

351 recognized due to limited access to marine habitats. Molecular approaches can be particularly
352 powerful in detecting cryptic species on the basis of their molecular divergence, and recent
353 molecular studies helped reveal the prevalence of cryptic species (See for example (Brasier et
354 al., 2016; Grant, Griffiths, Steinke, Wadley, & Linse, 2010)). This is particularly the case in
355 the Southern Ocean where numerous molecular studies have suggested the presence of cryptic
356 species (Baird, Miller, & Stark, 2011; Brasier et al., 2016; Grant et al., 2010; Wilson, Schrödl,
357 & Halanych, 2009) with areas such as the Scotia Arc representing potential hotspots of
358 cryptic diversity (Linse, Cope, Lörz, & Sands, 2007). The prevalence of invertebrate cryptic
359 species in the Southern Ocean is probably due to the cyclical variation of ice sheets extent in
360 Antarctica during repeated glacial and interglacial periods which could have fostered the
361 separation of population, promoting genetic divergence and allopatric cryptic speciation
362 (Clarke & Crame, 1989). During glacial periods, ice sheets extension is thought to have
363 forced marine species inhabiting the continental shelf to take refuge in the deep sea or in shelf
364 refugia, such as areas where permanent polynyas (Thatje et al., 2008) occurred. During glacial
365 maxima the decrease of gene flow between populations would have promoted reproductive
366 isolation and increased genetic variation between populations. Under glacial maximum
367 extreme environmental conditions, a potential increase of the diverging selective pressures on
368 behavioral and physiological characters rather than morphology (which could be under strong
369 stabilizing selection), could lead to a reduction of the morphological changes usually
370 associated with speciation (Fišer, Robinson, & Malard, 2018). In such a scenario, high levels
371 of divergence are expected on neutrally evolving genes as observed in this study. This process
372 might explain the high number of cryptic species reported in the Southern Ocean.

373 The concept of species is controversial in biology as there is no unique definition that can be
374 applied to all species under all circumstances (Mayden, 1997). Here, the application of
375 species delimitation methods (based on the genetic concept of species) using molecular tools
376 identified six highly divergent lineages indiscernible on the basis of their morphology and a
377 last morphologically divergent lineage. Therefore, this study show how the use of molecular
378 data can provide new insights into the nature of the genetic boundaries between species (Pante
379 et al., 2015). However, in the light of these findings we can wonder what do the lineages
380 highlighted in this study represent? Are they genuine different species? Here the use of
381 different criteria to delineate species based on reproductive isolation (biological concepts of
382 species) or on morphology (morphological concept of species) will probably lead to the
383 identification of a different number of species (Fišer et al., 2018). This is a good illustration of

384 the "species problem" and stress out the fact that the notion of species itself is an abstract
385 concept that should be questioned (De Queiroz, 2007). It has also important consequences in
386 term of the protection of the biodiversity because disparate groupings of species will in turn
387 result in different economic implications and conservation decisions (Frankham et al., 2012).

388

389 ***A remarkably fast turn over and low effective population sizes***

390 This study indicates that over time the different sectors of the Southern Ocean show a
391 remarkable turn over in term of phylogroup composition. For example, in less than a decade,
392 the Ross and East Weddell Sea phylogroups composition changed substantially (Fig 2a and
393 2c). A, B and F may or may not be sampled from one year to the next, whereas C, D and G
394 are always present but their proportions change drastically (Fig 2a and 2c). This observation
395 suggests that within the same geographical region, the real number of individual per
396 phylogroup varies considerably over time. One hypothesis that could explain these patterns is
397 the presence of strong marine currents (such as the ACC, Ross Gyre and Weddell Gyre)
398 mixing individuals and thus changing continuously their distribution across the Southern
399 Ocean over time. A similar mechanism has been evoked to explain the current diversity and
400 the distribution of sponges across the Southern Ocean (Downey, Griffiths, Linse, & Janussen,
401 2012).

402 Furthermore, within phylogroups, in a few years, the genetic makeup can change significantly.
403 For instance, the genetic composition of the phylogroup C and D changed totally between
404 1996 and 2008, respectively in the East Weddell Sea and Ross Sea (Fig. 2b, d). Such spatio-
405 temporal variations in term of genetic composition could also be attributed to marine currents
406 that constantly shuffle individuals between populations. Over time, the constant arrival of
407 individuals stemming from different populations continually modify the observed genetic
408 composition within the same geographical location.

409 Alternatively, a rapid divergence of the genetic pool by genetic drift, or any other population
410 processes, could also explain this rapid genetic turn over. The low effective population sizes
411 highlighted in this study ($Ne < 10$; Table 2) are also consistent with this pattern. Indeed, lower
412 values of *Ne* are associated with increased amount of "apparent" genetic drift. Furthermore,
413 isolated populations are expected to diverge inversely proportional to their effective
414 population sizes. Therefore, when the effective population sizes are extremely low,

415 populations can significantly diverge from each other in just a few generations (Hudson &
416 Coyne, 2002).

417 The simulations conducted in this study (Fig. 4) show that gene flow from an unsampled
418 reservoir should inflate the *Ne* values estimated with the method of Fu (Fu, 2001). Therefore,
419 due to the extremely low value of *Ne* observed, the hypothesis of a rapid divergence by
420 "apparent" genetic drift is favored here over the hypothesis of a rapid divergence mediated by
421 marine current continually modifying the genetic pool of the populations.

422 It is also important to bear in mind that our estimates of *Ne* depend directly on the number of
423 generations per year (Fu, 2001). In Table 2, we assumed a generation time of three year,
424 however if for example we assumed a generation time of 30 years, our estimates of *Ne* would
425 increase tenfold. In our case, depending on the assumed value of the generation time, the
426 interpretation of the *Ne* could change accordingly.

427 Overall, the *Ne* values estimated in this study for the *P. kerguelensis* and *F. mawsoni* are low.
428 However, they are highly abundant in the Southern Ocean (Hemery et al., 2013a; McClintock
429 & Pearse, 1987) which is indicative of a high census size. Such a discrepancy between the
430 census (*Nc*) and effective (*Ne*) population size is commonly observed in nature, especially for
431 marine species with large size (Filatov, 2019). However, the reasons behind it are not always
432 clear (Filatov, 2019; Palstra & Fraser, 2012). At least four biological phenomena could cause
433 *Nc* and *Ne* to be different in *P. kerguelensis* complex (with $Ne << Nc$). First, an unequal sex
434 ratio, i.e. an uneven number of males and females contributing to the next generation, lead the
435 rarer sex to have a greater effect on genetic drift which decreases *Ne* compare to *Nc*. Although
436 this hypothesis is plausible, to this day the sex ratio of *P. kerguelensis* and *F. mawsoni*
437 remains unknown. Furthermore, the sex-ratio distortion would need to be extreme to reach
438 such a low *Ne*. Second, a fluctuation of *Nc* over time can affect the ratio between *Ne* and *Nc*.
439 Indeed, an estimate of *Ne* over several generations is the harmonic mean of *Nc* over time.
440 Therefore, if *Nc* changes substantially over time, *Ne* is typically smaller than *Nc*. Fig. 2
441 indicates that the proportion of *P. kerguelensis* and *F. mawsoni* lineages can vary radically
442 from year to year and thus the fluctuation of *Nc* over time for each lineage can change
443 consequently leading *Ne* to be smaller than *Nc*. Third, the reproductive strategy of *P.*
444 *kerguelensis* and *F. mawsoni* can participate to reduce *Ne* compared to *Nc*. Indeed, when
445 individuals contribute unequally to the progeny of the next generation, a great proportion of
446 the next generation comes from a small number of individuals which reduces *Ne*. Due to its

447 mode of reproduction, *P. kerguelensis* is particularly incline to have a very reduced number of
448 individuals contributing disproportionately to the progeny. Reproduction occurs
449 synchronously around the months of November and December where males and females
450 spawn and due to the dispersion of gametes by currents, a very small proportion of individuals
451 have their ovocytes fertilized (McClintock & Pearse, 1987). Therefore, only a very small part
452 of the produced larvae contributes effectively to the gene pool. This phenomenon can
453 drastically reduce *Ne* compare to *Nc* and has been reported in different spawning species
454 (Lind, Evans, Knauer, Taylor, & Jerry, 2009). This mode of reproduction can also explain the
455 spatio-temporal genetic turn over mentioned previously. Lastly, due to its haploid and uni-
456 parental nature, the *Ne* of the mtDNA is known to be four times smaller than that of nuclear
457 DNA. Moreover, this effect can be exacerbated by the action of natural selection. Indeed,
458 natural selection can reduce *Ne* and the mtDNA is known to be under positive and negative
459 selection (Meiklejohn, Montooth, & Rand, 2007). Positive natural selection, for example, is
460 expected to reduce the genetic diversity leading to a low *Ne* (Gossmann, Woolfit, & Eyre-
461 Walker, 2011). Indeed, recurrent selective sweeps, related to positive mutations, will reduce
462 the genetic diversity by hitchhiking resulting in a lower *Ne* compared to *Nc*. In this regard,
463 Piganeau & Eyre-Walker (2009) found a strong negative correlation between *Ne* and the
464 strength of the natural selection operating on the mtDNA on non-synonymous mutations. In
465 addition, the non-recombining nature of the mtDNA is expected to make this effect even
466 stronger. There are accumulating evidences suggesting that positive natural selection is acting
467 on echinoderm mitogenomes (Castellana, Vicario, & Saccone, 2011). For example, the strong
468 bias of codon usage observed in the mtDNA of the crinoid species *Florometra serratissima*
469 compared to many other echinoderms, is also in favor of the idea that some sort of natural
470 selection is occurring, though the mechanism behind it is still not fully understood (Scouras &
471 Smith, 2001).

472

473 ***Tempo of speciation: Promachocrinus kerguelensis challenges the***
474 ***biodiversity pump hypothesis.***

475 The mutation rate estimated in this study is approximatively equal to 10^{-5} mutations/site/year
476 and is based on population genetics data. Previous estimate of the mutation rate based also on
477 the COI applied to closely related species (Lessios et al., 1999) used a phylogeny based
478 approach and yielded a mutation rate of roughly 3 order of magnitude lower (≈ 10^{-8}

479 mutations/site/year). Such a discrepancy between mutation rates derived from population data
480 and phylogeny has been already documented (Burridge, Craw, Fletcher, & Waters, 2008;
481 Madrigal et al., 2012) and is called "time dependency of molecular rates" (Ho et al., 2011; Ho,
482 Phillips, Cooper, & Drummond, 2005). For example, in human, the mitochondrial D-loop
483 mutation rate derived from pedigree approaches produced an average value of 8.10^{-7}
484 mutations/site/year whereas phylogenetic estimates yielded value of 2.10^{-8} mutations/site/year
485 (Santos et al., 2005). Likewise, in fishes, Burridge (Burridge et al., 2008) observed a mutation
486 rate of $\approx 10^{-7}$ using a population based approach and $\approx 10^{-8}$ mutations/site/year from a
487 phylogenetic estimates. The potential causes of the "time scale dependency" are reviewed in
488 Ho *et al.* (2011), but one of the main reason behind it is that population genetics based
489 calibrations lead to estimates reflecting the spontaneous mutation rates, whereas phylogeny
490 based calibrations reflect the substitution rates (i.e. fixed mutations). Therefore, because
491 purifying selection remove the vast majority of the spontaneous deleterious mutations, which
492 constitute a large proportion of them, the spontaneous mutation rate is higher than the
493 substitution rate. Consequently, the rates of molecular evolution decrease with the age of the
494 calibration used to estimate them and it is invalid to assume that a unique molecular clock
495 applies over all time scales (Ho et al., 2005; 2011). A major implication of time dependency
496 is that estimates of recent population divergence times will require re-estimation (Burridge et
497 al., 2008). Therefore, many studies suggesting population isolation related to the Pleistocene
498 glaciations might need to convert these time estimates to more recent ones (i.e. last glacial
499 maximum or Holocene).

500 Furthermore, the large variation of the mutation rates across the mtDNA regions and between
501 different lineages (Nabholz, Glemin, & Galtier, 2009), leads the mtDNA to strongly deviate
502 from the molecular clock assumption, which is generally assumed in phylogenetic
503 reconstruction. Therefore, estimates of mtDNA mutation rates relying on phylogenetic
504 approach should be taken with caution (Nabholz et al., 2009; Nabholz, Glemin, & Galtier,
505 2008). Conversely, the mutation rate estimated in this study are quite robust as they are
506 model-free ((Fu, 2001) ; See also Material and Methods). In the case of *P. kerguelensis* and *F.*
507 *mawsoni*, the different mutation rates estimated have important consequences on the age of
508 the separation of the different lineages (Fig. 4). Indeed, the estimate from Lessios *et al.* (1999)
509 suggest that the split between the different lineages would have occurred in the last million
510 year (Fig. 4), which is congruent with the hypothesis of the biodiversity pump suggesting that
511 most of the speciation events would have occurred during the Quaternary cycles (Clarke et al.,

1992; Clarke & Crame, 1989). Conversely, with our estimate the separation would have occurred in the last 1000 years, implying an extremely recent and rapid diversification for *P. kerguelensis* and *F. mawsoni*.

Explosive radiations have been reported for several taxa (Mahler, Ingram, Revell, & Losos, 2013; Moore & Robertson, 2014; Muschick, Indermaur, & Salzburger, 2012) and are, in general, associated with the Pleistocene glacial cycles (Hawlitschek et al., 2012). In contrast, using the molecular rate estimated in this study, in our case the diversification would have happened during the Holocene. Such a remarkably rapid diversification has been rarely recorded in nature (but see Muschick et al., 2012; Peccoud, Simon, McLaughlin, & Moran, 2009). Furthermore, most of rapid radiations reported so far are adaptive and associated to morphological novelties that allow taxa to exploit separate niches. Taxa involved in rapid diversification are generally ecologically/morphologically highly differentiated (Losos & Miles, 2002). Here, most of the lineages display no obvious morphological differences (except for *Florometra*) or niche differentiation. As a consequence, the radiation within the *P. kerguelensis* complex is probably mainly nonadaptive (Rundell & Price, 2009) or adaptive but related to characters that are challenging to distinguish/observe (physiology or behavior). In this context, it is worth noting that structural genomic changes could also drive taxa to rapid adaptive (Kirkpatrick & Barton, 2006) and nonadaptive (Rowe, Aplin, Baverstock, & Moritz, 2011; Rundell & Price, 2009) radiation and may represent the mechanism underlying the diversification of the *P. kerguelensis* complex.

Finally, the low effective population sizes estimated (Table 2) are congruent with the rapid diversification rate observed. Indeed, Hudson and Coyne (2002) showed that for mtDNA, under the isolation model, two lineages would take between 2 and 3*Ne* generations to become reciprocally monophyletic by lineage sorting. Therefore, using the low effective population size estimated in this study (Table 2), it would take a few hundreds of years for two isolated taxa to reach complete reciprocal monophyly. This result is concordant with the divergence observed in this study and contributes to invalidate the Antarctic “biodiversity pump” for the *Promachocrinus* complex.

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Tables and figures

Table 1: Pairwise F-st and the probability from the Hudson et al. (1992) tests for all phylogroups with 2 samples of 10+ sequences within the same location.

Below diagonal: Fst. Above diagonal: probability of pamixia using the Hudson test.

EWS										RS		
C				E			G			D		
n=	39	37	24		10	11		14	44		27	26
	1996	2000	2004		2000	2004		2004	2005		2004	2008
1996	-	0.035	0.004	2000	-	0.32	2004	-	0.40	2004	-	0.0002
2000	0.05	-	$<10^{-4}$	2004	0.04	-	2005	0.01	-	2008	0.21	-
2004	0.19	0.47	-									

833 **Table 2: Estimation of mutation rate and effective population size**

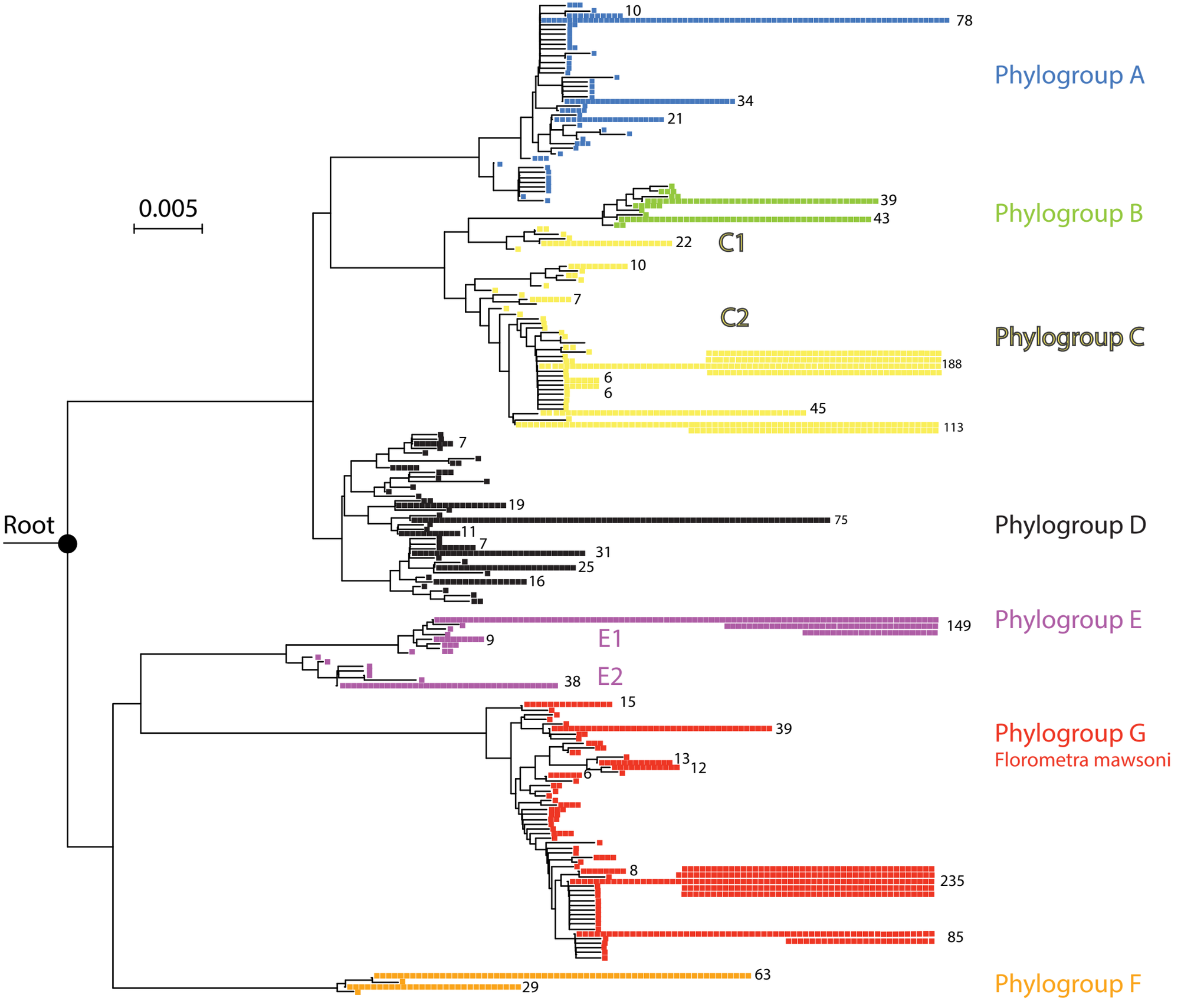
834 Summary table of effective population sizes (*Ne* in numbers of individuals) and mutation
835 rates (μ per base pair per year) for each phylogroup estimated by the Fu (2001) method. We
836 also report the years and the number sequences used for each estimation.

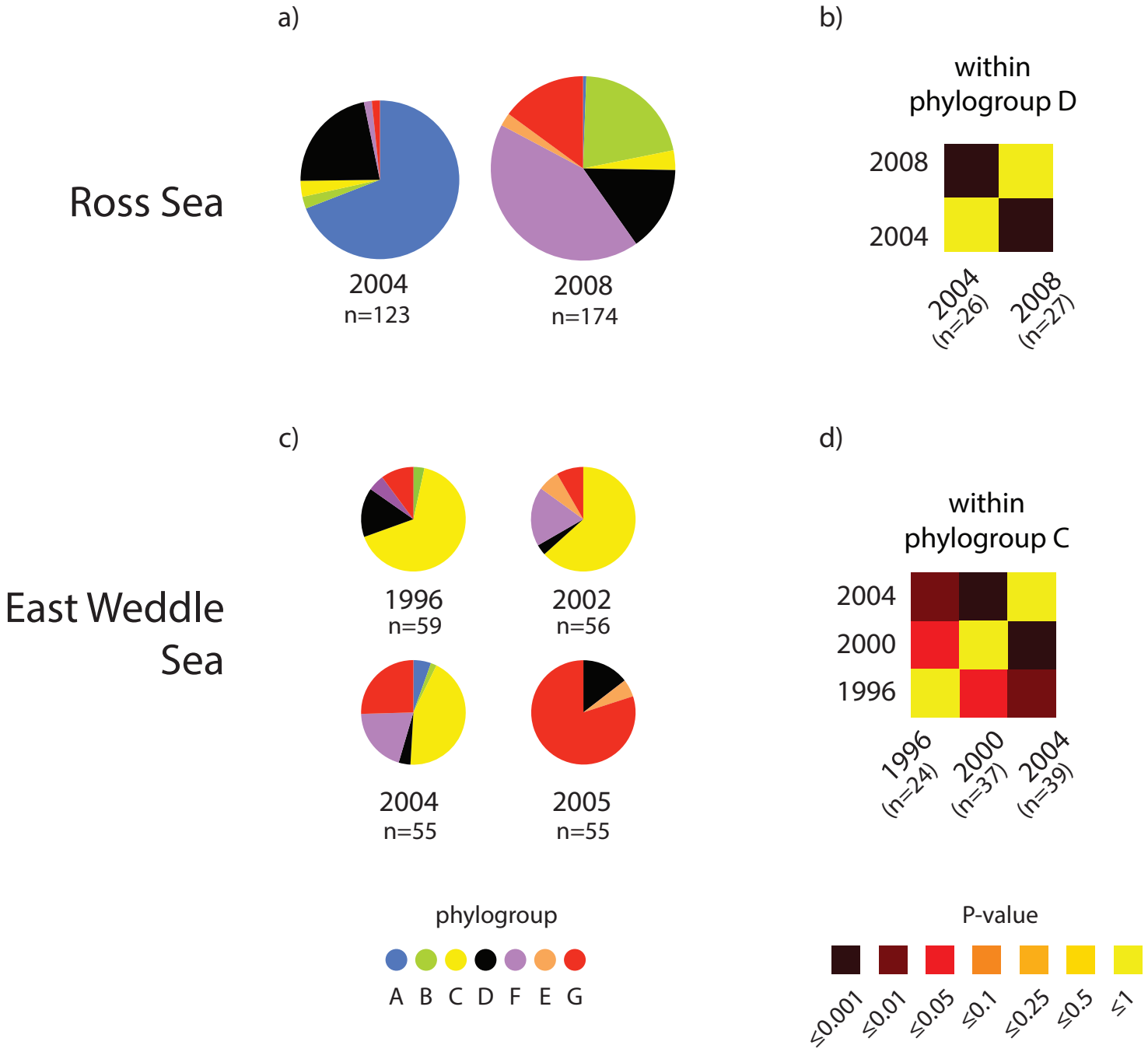
Location	EWS							RS	
Phylogroup	C			E		G		D	
n=	39	37	24	10	11	14	44	27	26
Dates	1996	2000	2004	2000	2004	2004	2005	2004	2008
μ	$1.9\ 10^{-5}$			$2.7\ 10^{-5}$		$3.5\ 10^{-4}$		$4.2\ 10^{-4}$	
Ne	4.0			7.0		1.7		2.6	

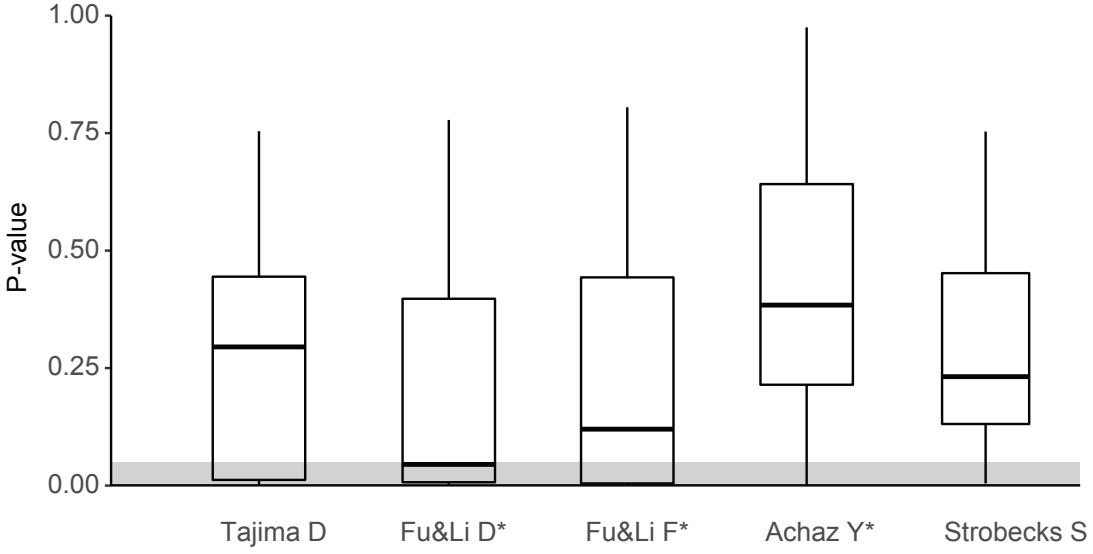
837

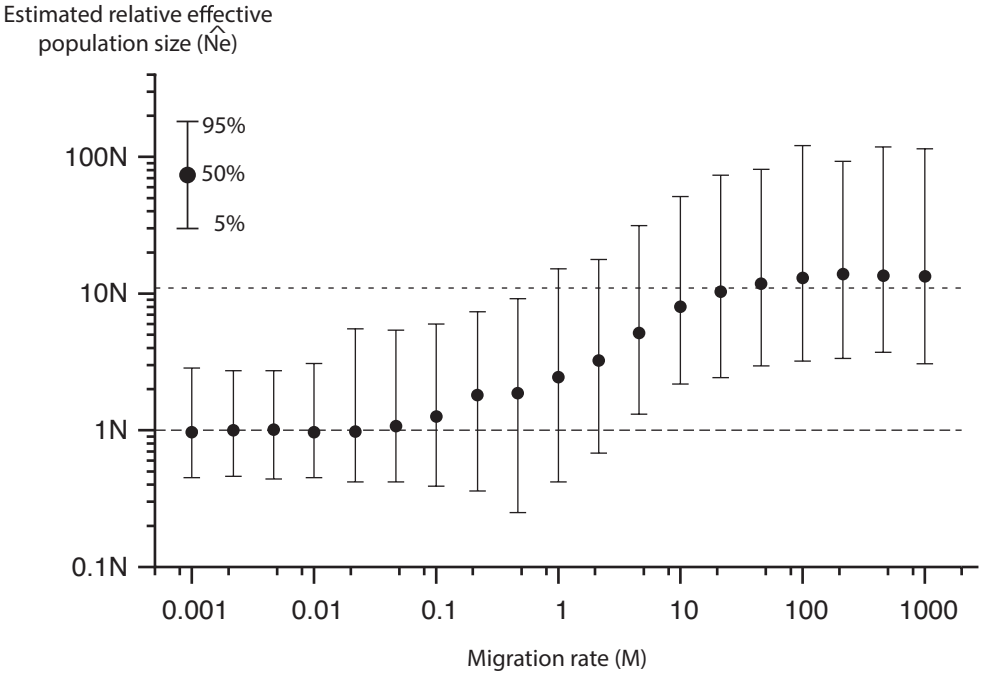
838

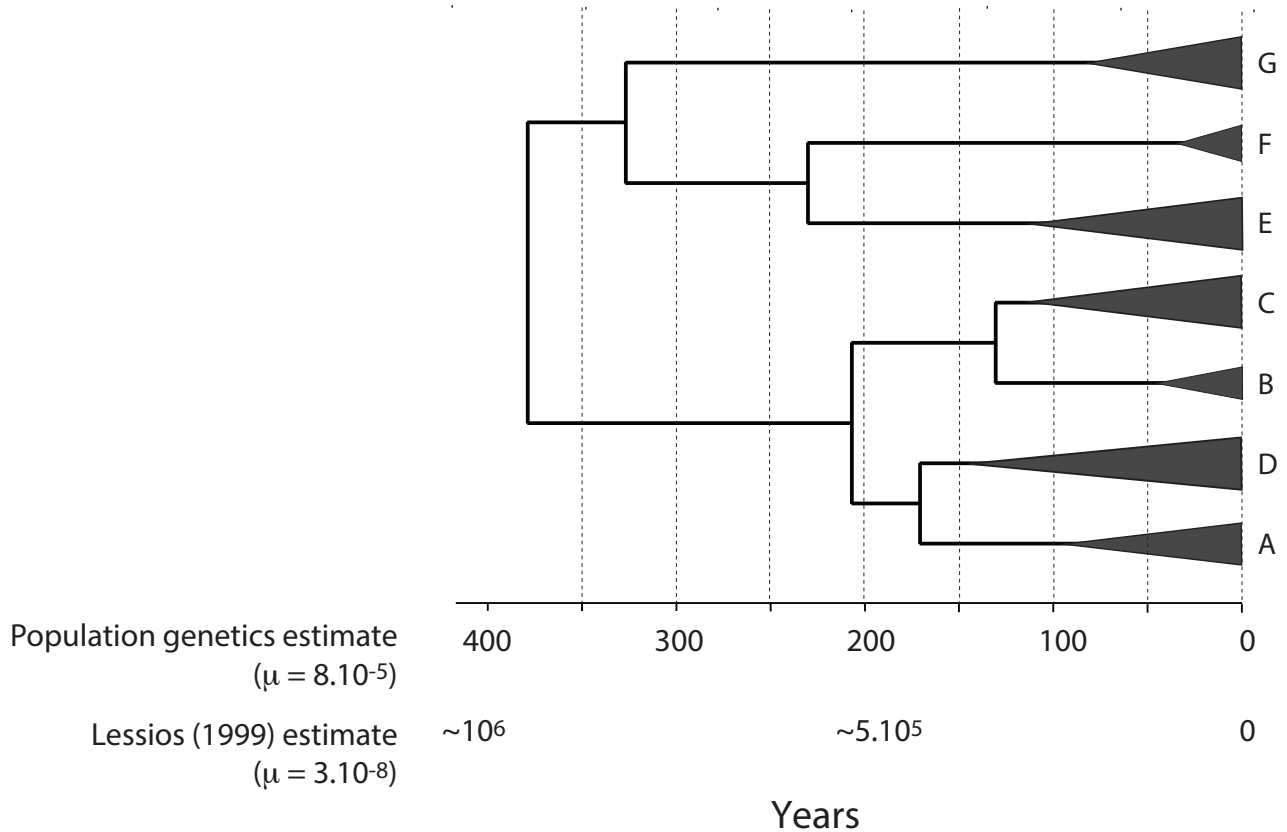
839 **Fig. 1:** Phylogenetic representation of the 1797 COI sequences from *P. kerguelensis* and
840 *F. mawsoni*. The tree was inferred using BIONJ on the unique sequences. Each square
841 represents one sequence. The phylogroups are the ones used in the analysis (mostly ABGD
842 groups) and both supplementary splits proposed by PTP are indicated as C1/C2 and E1/E2.
843 The colors represent the different phylogroups considered in this study. The boxes and the
844 numbers refer to the number of individuals carrying the haplotype.
845
846 **Fig. 2:** Genetic turn over at the RS (a and b) and EWS (c and d) locations. The pie charts (a
847 and c) represent the number of phylogroups at different years in the locations, with the same
848 colors code as in Figure 1. The heatmaps (b and d) represents P-values of the Hudson et *al.*
849 (1992) tests for genetic structure within a phylogroup of each location.
850
851 **Fig. 3:** Distribution of p-values for different neutrality tests on the 1,624 sequences tested for
852 group with at least 10 sequences. From left to right: Tajima D (1983), Fu and Li D* (1993),
853 Fu and Li F* (1993), Achaz Y* (2008) and Strobeck S (1987).
854
855 **Fig. 4:** Simulation of migration effect on Ne estimation.
856
857 **Fig. 5:** Divergence time of the different phylogroup of the *P. kerguelensis* complex. The
858 depth of the triangles represents the maximum diversity within each phylogroup. The time is
859 indicated in years before present. The timescale on the top is assuming a mutation rate of
860 8.10^{-5} mutation/bp/year. The timescale on the bottom is assuming a mutation rate of 8.10^{-3}
861 mutation/bp/year.


0.005
Root
Phylogroup A
Phylogroup B
Phylogroup C
Phylogroup D
Phylogroup E
Phylogroup G
Florometra mawsoni
Phylogroup F
C1
C2
E1
E2
10
78
34
21
39
43
22
10
7
188
6
6
45
113
7
19
75
11
7
31
25
16
149
9
38
15
39
13
12
6
8
235
85
63
29


a)
b)
Ross Sea
2004
n=123
2008
n=174
within
phylogroup D
2008
2004
2004
(n=26)
2008
(n=27)
c)
d)
East Weddle
Sea
1996
n=59
2002
n=56
2004
n=55
2005
n=55
within
phylogroup C
2004
2000
1996
1996
(n=24)
2000
(n=37)
2004
(n=39)
phylogroup
A B C D F E G
P-value
≤0.001
≤0.01
≤0.05
≤0.1
≤0.25
≤0.5
≤1


1.00
0.75
0.50
0.25
0.00
P-value
Tajima D
Fu&Li D*
Fu&Li F*
Achaz Y*
Strobecks S


Estimated relative effective population size ($\hat{N}e$)
95%
50%
5%
100N
10N
1N
0.1N
0.001
0.01
0.1
1
10
100
1000
Migration rate (M)


G
F
E
C
B
D
A
Population genetics estimate
(μ = 8.10-5)
400
300
200
100
0
Lessios (1999) estimate
(μ = 3.10-8)
~10^6
~5.10^5
0
Years