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 using 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:

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 Some adults of *P. kerguelensis* have also been observed swimming (Eléaume, unpublished observations), and some adults of *F. mawsoni*, though never observed swimming in situ, have shown this ability in tanks (Eléaume, unpublished observations).

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

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

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

149 ***Assessing the temporal structure within phylogroups***

150 We characterized the temporal structures of our samples in pairwise comparisons using both
151 fixation index F_{ST} as well as a non-parametric structure test based on K^*s by Hudson *et al.*
152 (1992). All p-values were estimated using permutations as described in Hudson *et al.* (1992).
153 A web-interface for the latter can be found at http://wwwabi.snv.jussieu.fr/public/mpweb/.

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

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

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

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

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

173

174 ***Polymorphisms analysis***

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

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

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

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

227 Other locations display a single sampling event (AS, DS, TA) or reduced number of
228 sequences over several sampling events (e.g. SAW: 2 sequences in 2000, 11 in 2002, 7 in
229 2006 and 68 in 2009), and cannot be used to robustly assess the stability of phylogroup
230 relative abundance.

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

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

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

252 ***Polymorphism analysis***

253 For samples of 10 sequences or more (with the same year and same location), we have
254 analyzed the characteristics of segregating polymorphisms using four neutrality tests:
255 Tajima's D (Tajima, 1989), Fu and Li's F* and D* (Fu & Li, 1993), Achaz's Y* (Achaz,
256 2008) and Strobeck P-values (Strobeck, 1987). Results (Fig. 3) show almost no deviation
257 from the standard neutral model, except for the Fu and Li's F* and D*. This statistics points
258 to a clear excess of singletons. However, singletons likely contain sequencing errors that
259 strongly skew these statistics (Achaz, 2008). The Y statistics that ignores singletons exhibits a
260 distribution that is very close to the one expected under the standard neutral model. We
261 therefore conclude that the observed polymorphisms suggest that basic assumptions of the
262 standard neutral model (i.e. constant population size, no migration, no structure and no
263 selection) cannot be rejected for these crinoid phylogroups.

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

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

275 ***Phylogroups divergence***

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

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

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

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

383

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

385 This study indicates that over time the different sectors of the Southern Ocean show a
386 remarkable turn over in term of phylogroup composition. For example, in less than a decade,
387 the Ross and East Weddell Sea phylogroups composition changed substantially (Fig 2a and
388 2c). A, B and F may or may not be sampled from one year to the next, whereas C, D and G
389 are always present but their proportions change drastically (Fig 2a and 2c). This observation
390 suggests that within the same geographical region, the real number of individual per
391 phylogroup varies considerably over time. One hypothesis that could explain these patterns is
392 the presence of strong marine currents (such as the ACC, Ross Gyre and Weddell Gyre)
393 mixing individuals and thus changing continuously their distribution across the Southern
394 Ocean over time. A similar mechanism has been evoked to explain the current diversity and
395 the distribution of sponges across the Southern Ocean (Downey, Griffiths, Linse, & Janussen,
396 2012).

397 Furthermore, within phylogroups, in a few years, the genetic makeup can change significantly.
398 For instance, the genetic composition of the phylogroup C and D changed totally between
399 1996 and 2008, respectively in the East Weddell Sea and Ross Sea (Fig. 2b, d). Such spatio-
400 temporal variations in term of genetic composition could also be attributed to marine currents
401 that constantly shuffle individuals between populations. Over time, the constant arrival of
402 individuals stemming from different populations continually modify the observed genetic
403 composition within the same geographical location.

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

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

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

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

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

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

467

468 ***Tempo of speciation: Promachocrinus kerguelensis challenges the***
469 ***biodiversity pump hypothesis.***

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

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

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

Achaz, G. (2008). Testing for neutrality in samples with sequencing errors. *Genetics*, *179*(3), 1409–1424. http://doi.org/10.1534/genetics.107.082198

Allcock, A. L., & Strugnell, J. M. (2012). Southern Ocean diversity: new paradigms from molecular ecology. *Trends in Ecology & Evolution*, *27*(9), 520–528. http://doi.org/10.1016/j.tree.2012.05.009

Baird, H. P., Miller, K. J., & Stark, J. S. (2011). Evidence of hidden biodiversity, ongoing speciation and diverse patterns of genetic structure in giant Antarctic amphipods. *Molecular Ecology*, *20*(16), 3439–3454. http://doi.org/10.1111/j.1365-294X.2011.05173.x

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., et al. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*(3), 148–155. http://doi.org/10.1016/j.tree.2006.11.004

Brasier, M. J., Wiklund, H., Neal, L., Jeffreys, R., Linse, K., Ruhl, H., & Glover, A. G. (2016). DNA barcoding uncovers cryptic diversity in 50% of deep-sea Antarctic polychaetes. *Royal Society Open Science*, *3*(11), 160432. http://doi.org/10.1098/rsos.160432

Burridge, C. P., Craw, D., Fletcher, D., & Waters, J. M. (2008). Geological dates and molecular rates: fish DNA sheds light on time dependency. *Molecular Biology and Evolution*, *25*(4), 624–633. http://doi.org/10.1093/molbev/msm271

Castellana, S., Vicario, S., & Saccone, C. (2011). Evolutionary patterns of the mitochondrial genome in Metazoa: exploring the role of mutation and selection in mitochondrial protein coding genes. *Genome Biology and Evolution*, *3*(3), 1067–1079. http://doi.org/10.1093/gbe/evr040

Chown, S. L., Clarke, A., Fraser, C. I., Cary, S. C., Moon, K. L., & McGeoch, M. A. (2015). The changing form of Antarctic biodiversity. *Nature*, *522*(7557), 431–438. http://doi.org/10.1038/nature14505

Clark, A. H. (1921). A monograph of the existing crinoids. *Bulletin of the United States National Museum*, *1*(82), 795–949. http://doi.org/10.5479/si.03629236.82.2

Clarke, A., & Crame, J. A. (1989). The origin of the Southern Ocean marine fauna. *Geological Society, London, Special Publications*, *47*(1), 253–268. http://doi.org/10.1144/GSL.SP.1989.047.01.19

Clarke, A., Crame, J. A., Stromberg, J. O., & Barker, P. F. (1992). The Southern Ocean Benthic Fauna and Climate Change: A Historical Perspective [and Discussion]. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *338*(1285), 299–309. http://doi.org/10.1098/rstb.1992.0150

Cornils, A., & Held, C. (2014). Evidence of cryptic and pseudocryptic speciation in the *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida). *Frontiers in Zoology*, *11*(1), 19. http://doi.org/10.1186/1742-9994-11-19

Davies, B. J., Hambrey, M. J., Smellie, J. L., Carrivick, J. L., & Glasser, N. F. (2012). Antarctic Peninsula Ice Sheet evolution during the Cenozoic Era. *Quaternary Science Reviews*, *31*, 30–66. http://doi.org/10.1016/j.quascirev.2011.10.012

De Queiroz, K. (2007). Species Concepts and Species Delimitation. *Systematic Biology*, *56*(6), 879–886. http://doi.org/10.1080/10635150701701083

DeBiasse, M. B., & Hellberg, M. E. (2015). Discordance between morphological and molecular species boundaries among Caribbean species of the reef sponge Callyspongia. *Ecology and Evolution*, *5*(3), 663–675. http://doi.org/10.1002/ece3.1381

Downey, R. V., Griffiths, H. J., Linse, K., & Janussen, D. (2012). Diversity and distribution patterns in high southern latitude sponges. *PloS One*, *7*(7), e41672.

http://doi.org/10.1371/journal.pone.0041672
Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*(8), 1969–1973. http://doi.org/10.1093/molbev/mss075
Eastman, J. T., & McCune, A. R. (2000). Fishes on the Antarctic continental shelf: evolution of amarine species flock?*. *Journal of Fish Biology*, *57*(sa), 84–102. http://doi.org/10.1111/j.1095-8649.2000.tb02246.x
Eléaume, M. (2006). Approche morphométrique de la variabilité phénotipique : conséquences systématiques et évolutives : application aux crinoïdes actuels (Crinoidea : Echinodermata). *Www.Theses.Fr*.
Eléaume, M., Hemery, L. G., Roux, M., & Améziane, N. (2014). Southern Ocean Crinoids. In *Biogeographic Atlas of the Southern Ocean* (pp. 208–212). Cambridge.
Felsenstein, J. (1989). PHYLIP - Phylogeny Inference Package (version 3.2). *Cladistics*, (5), 164–166.
Filatov, D. A. (2019). Extreme Lewontin's Paradox in Ubiquitous Marine Phytoplankton Species. *Molecular Biology and Evolution*, *36*(1), 4–14. http://doi.org/10.1093/molbev/msy195
Fišer, C., Robinson, C. T., & Malard, F. (2018). Cryptic species as a window into the paradigm shift of the species concept. *Molecular Ecology*, *27*(3), 613–635. http://doi.org/10.1111/mec.14486
Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*(5), 294–299.
Frankham, R., Ballou, J. D., Dudash, M. R., Eldridge, M. D. B., Fenster, C. B., Lacy, R. C., et al. (2012). Implications of different species concepts for conserving biodiversity. *Biological Conservation*, *153*, 25–31. http://doi.org/10.1016/j.biocon.2012.04.034
Fu, Y. X. (2001). Estimating mutation rate and generation time from longitudinal samples of DNA sequences. *Molecular Biology and Evolution*, *18*(4), 620–626. http://doi.org/10.1093/oxfordjournals.molbev.a003842
Fu, Y. X., & Li, W. H. (1993). Statistical tests of neutrality of mutations. *Genetics*, *133*(3), 693–709.
Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution*, *14*(7), 685–695. http://doi.org/10.1093/oxfordjournals.molbev.a025808
Gossmann, T. I., Woolfit, M., & Eyre-Walker, A. (2011). Quantifying the variation in the effective population size within a genome. *Genetics*, *189*(4), 1389–1402. http://doi.org/10.1534/genetics.111.132654
Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, *27*(2), 221–224. http://doi.org/10.1093/molbev/msp259
Grant, R. A., Griffiths, H. J., Steinke, D., Wadley, V., & Linse, K. (2010). Antarctic DNA barcoding; a drop in the ocean? *Polar Biology*, *34*(5), 775–780. http://doi.org/10.1007/s00300-010-0932-7
Griffiths, H. J. (2010). Antarctic marine biodiversity--what do we know about the distribution of life in the Southern Ocean? *PloS One*, *5*(8), e11683. http://doi.org/10.1371/journal.pone.0011683
Griffiths, H. J., Barnes, D. K. A., & Linse, K. (2009). Towards a generalized biogeography of the Southern Ocean benthos. *Journal of Biogeography*, *36*(1), 162–177. http://doi.org/10.1111/j.1365-2699.2008.01979.x
Hawlitschek, O., Hendrich, L., Espeland, M., Toussaint, E. F. A., Genner, M. J., & Balke, M.

(2012). Pleistocene climate change promoted rapid diversification of aquatic invertebrates in Southeast Australia. *BMC Evolutionary Biology*, *12*(1), 142. http://doi.org/10.1186/1471-2148-12-142

Heimeier, D., Lavery, S., & Sewell, M. A. (2010). Molecular species identification of *Astrotoma agassizii* from planktonic embryos: further evidence for a cryptic species complex. *The Journal of Heredity*, *101*(6), 775–779. http://doi.org/10.1093/jhered/esq074

Hemery, L. G., Améziane, N., & Eléaume, M. (2013a). Circumpolar dataset of sequenced specimens of Promachocrinus kerguelensis (Echinodermata, Crinoidea). *ZooKeys*, *315*(60), 55–64. http://doi.org/10.3897/zookeys.315.5673

Hemery, L. G., Eléaume, M., Roussel, V., Améziane, N., Gallut, C., Steinke, D., et al. (2012). Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis* (Echinodermata). *Molecular Ecology*, *21*(10), 2502–2518. http://doi.org/10.1111/j.1365-294X.2012.05512.x

Hemery, L. G., Roux, M., Améziane, N., & Eléaume, M. (2013b). High-resolution crinoid phyletic inter-relationships derived from molecular data. *Cahiers De Biologie Marine*, *54*(4), 511–525.

Ho, S. Y. W., Lanfear, R., Bromham, L., Phillips, M. J., Soubrier, J., Rodrigo, A. G., & Cooper, A. (2011). Time-dependent rates of molecular evolution. *Molecular Ecology*, *20*(15), 3087–3101. http://doi.org/10.1111/j.1365-294X.2011.05178.x

Ho, S. Y. W., Phillips, M. J., Cooper, A., & Drummond, A. J. (2005). Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution*, *22*(7), 1561–1568. http://doi.org/10.1093/molbev/msi145

Hudson, R. R., & Coyne, J. A. (2002). Mathematical consequences of the genealogical species concept. *Evolution*, *56*(8), 1557–1565.

Hudson, R. R., Boos, D. D., & Kaplan, N. L. (1992). A statistical test for detecting geographic subdivision. *Molecular Biology and Evolution*, *9*(1), 138–151. http://doi.org/10.1093/oxfordjournals.molbev.a040703

Kirkpatrick, M., & Barton, N. (2006). Chromosome inversions, local adaptation and speciation. *Genetics*, *173*(1), 419–434. http://doi.org/10.1534/genetics.105.047985

Knowlton, N. (1993). Sibling Species in the Sea. *Annual Review of Ecology and Systematics*, *24*(1), 189–216. http://doi.org/10.1146/annurev.ecolsys.24.1.189

Knowlton, N. (2000). Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia*, *420*(1), 73–90. http://doi.org/10.1023/A:1003933603879

Lecointre, G., Améziane, N., Boisselier, M.-C., Bonillo, C., Busson, F., Causse, R., et al. (2013). Is the species flock concept operational? The Antarctic shelf case. *PloS One*, *8*(8), e68787. http://doi.org/10.1371/journal.pone.0068787

Lessios, H. A., Kessing, B. D., Robertson, D. R., & Paulay, G. (1999). Phylogeography of the Pantropical Sea Urchin Eucidaris in Relation to Land Barriers and Ocean Currents. *Evolution*, *53*(3), 806. http://doi.org/10.2307/2640720

Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*(11), 1451–1452. http://doi.org/10.1093/bioinformatics/btp187

Lind, C. E., Evans, B. S., Knauer, J., Taylor, J. J. U., & Jerry, D. R. (2009). Decreased genetic diversity and a reduced effective population size in cultured silver-lipped pearl oysters (*Pinctada maxima*). *Aquaculture*, *286*(1-2), 12–19. http://doi.org/10.1016/j.aquaculture.2008.09.009

Linse, K., Cope, T., Lörz, A.-N., & Sands, C. (2007). Is the Scotia Sea a centre of Antarctic marine diversification? Some evidence of cryptic speciation in the circum-Antarctic

bivalve *Lissarca notorcadensis* (Arcoidea: Philobryidae). *Polar Biology*, *30*(8), 1059–1068. http://doi.org/10.1007/s00300-007-0265-3

Losos, J. B., & Miles, D. B. (2002). Testing the hypothesis that a clade has adaptively radiated: iguanid lizard clades as a case study. *The American Naturalist*, *160*(2), 147–157. http://doi.org/10.1086/341557

Madrigal, L., Posthumously, L. C., Melendez-Obando, M., Villegas-Palma, R., Barrantes, R., Raventos, H., et al. (2012). High mitochondrial mutation rates estimated from deep-rooting Costa Rican pedigrees. *American Journal of Physical Anthropology*, *148*(3), 327–333. http://doi.org/10.1002/ajpa.22052

Mahler, D. L., Ingram, T., Revell, L. J., & Losos, J. B. (2013). Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science*, *341*(6143), 292–295. http://doi.org/10.1126/science.1232392

Mayden, R. L. (1997). A hierarchy of species concepts: the denouement in the saga of the species problem.

McClintock, J. B., & Pearse, J. S. (1987). Reproductive biology of the common antarctic crinoid *Promachocrinus kerguelensis* (Echinodermata: Crinoidea). *Marine Biology*, *96*(3), 375–383. http://doi.org/10.1007/BF00412521

Meiklejohn, C. D., Montooth, K. L., & Rand, D. M. (2007). Positive and negative selection on the mitochondrial genome. *Trends in Genetics : TIG*, *23*(6), 259–263. http://doi.org/10.1016/j.tig.2007.03.008

Michener, C. D., & Sokal, R. R. (1957). A quantitative approach to a problem in classification. *Evolution*, *11*(2), 130–162. http://doi.org/10.1111/j.1558-5646.1957.tb02884.x

Moore, W., & Robertson, J. A. (2014). Explosive adaptive radiation and extreme phenotypic diversity within ant-nest beetles. *Current Biology : CB*, *24*(20), 2435–2439. http://doi.org/10.1016/j.cub.2014.09.022

Muschick, M., Indermaur, A., & Salzburger, W. (2012). Convergent evolution within an adaptive radiation of cichlid fishes. *Current Biology : CB*, *22*(24), 2362–2368. http://doi.org/10.1016/j.cub.2012.10.048

Nabholz, B., Glemin, S., & Galtier, N. (2008). Strong variations of mitochondrial mutation rate across mammals--the longevity hypothesis. *Molecular Biology and Evolution*, *25*(1), 120–130. http://doi.org/10.1093/molbev/msm248

Nabholz, B., Glemin, S., & Galtier, N. (2009). The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology*, *9*(1), 54. http://doi.org/10.1186/1471-2148-9-54

Palstra, F. P., & Fraser, D. J. (2012). Effective/census population size ratio estimation: a compendium and appraisal. *Ecology and Evolution*, *2*(9), 2357–2365. http://doi.org/10.1002/ece3.329

Palumbi, S. R. (1994). Genetic Divergence, Reproductive Isolation, and Marine Speciation. *Annual Review of Ecology and Systematics*, *25*(1), 547–572. http://doi.org/10.1146/annurev.ecolsys.25.1.547

Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S. C., Boisselier, M. C., & Samadi, S. (2015). Use of RAD sequencing for delimiting species. *Heredity*, *114*(5), 450–459. http://doi.org/10.1038/hdy.2014.105

Peccoud, J., Simon, J.-C., McLaughlin, H. J., & Moran, N. A. (2009). Post-Pleistocene radiation of the pea aphid complex revealed by rapidly evolving endosymbionts. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(38), 16315–16320. http://doi.org/10.1073/pnas.0905129106

Pfenninger, M., & Schwenk, K. (2007). Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, *7*(1), 121. http://doi.org/10.1186/1471-2148-7-121

Piganeau, G., & Eyre-Walker, A. (2009). Evidence for variation in the effective population size of animal mitochondrial DNA. *PloS One*, *4*(2), e4396. http://doi.org/10.1371/journal.pone.0004396

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., et al. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, *55*(4), 595–609.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2011). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, *21*(8), 1864–1877. http://doi.org/10.1111/j.1365-294X.2011.05239.x

Rogers, A. D. (2007). Evolution and biodiversity of Antarctic organisms: a molecular perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1488), 2191–2214. http://doi.org/10.1098/rstb.2006.1948

Rogers, A. D. (2012). Evolution and Biodiversity of Antarctic Organisms. In *Antarctic Ecosystems* (Vol. 187, pp. 417–467). Chichester, UK: John Wiley & Sons, Ltd. http://doi.org/10.1002/9781444347241.ch14

Rowe, K. C., Aplin, K. P., Baverstock, P. R., & Moritz, C. (2011). Recent and Rapid Speciation with Limited Morphological Disparity in the Genus Rattus. *Systematic Biology*, *60*(2), 188–203. http://doi.org/10.1093/sysbio/syq092

Rundell, R. J., & Price, T. D. (2009). Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology & Evolution*, *24*(7), 394–399. http://doi.org/10.1016/j.tree.2009.02.007

Santos, C., Montiel, R., Sierra, B., Bettencourt, C., Fernandez, E., Alvarez, L., et al. (2005). Understanding differences between phylogenetic and pedigree-derived mtDNA mutation rate: a model using families from the Azores Islands (Portugal). *Molecular Biology and Evolution*, *22*(6), 1490–1505. http://doi.org/10.1093/molbev/msi141

Scouras, A., & Smith, M. J. (2001). A novel mitochondrial gene order in the crinoid echinoderm *Florometra serratissima*. *Molecular Biology and Evolution*, *18*(1), 61–73. http://doi.org/10.1093/oxfordjournals.molbev.a003720

Speel, J. A., & Dearborn, J. H. (1983). Comatulid Crinoids From R/V Eltanin Cruises In The Southern Ocean. In *Biology of the Antarctic Seas XIII* (Vol. 38, pp. 1–60). Washington, D. C.: American Geophysical Union. http://doi.org/10.1029/AR038p0001

Strobeck, C. (1987). Average number of nucleotide differences in a sample from a single subpopulation: a test for population subdivision. *Genetics*, *117*(1), 149–153.

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, *105*(2), 437–460.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*(3), 585–595.

Thatje, S., Hillenbrand, C.-D., & Larter, R. (2005). On the origin of Antarctic marine benthic community structure. *Trends in Ecology & Evolution*, *20*(10), 534–540. http://doi.org/10.1016/j.tree.2005.07.010

Thatje, S., Hillenbrand, C.-D., Mackensen, A., & Larter, R. (2008). Life hung by a thread: endurance of Antarctic fauna in glacial periods. *Ecology*, *89*(3), 682–692.

Thornhill, D. J., Mahon, A. R., Norenburg, J. L., & Halanych, K. M. (2008). Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm Parborlasia corrugatus (Nemertea: Lineidae). *Molecular Ecology*, *17*(23), 5104–5117. http://doi.org/10.1111/j.1365-294X.2008.03970.x

Tripati, A., Backman, J., Elderfield, H., & Ferretti, P. (2005). Eocene bipolar glaciation associated with global carbon cycle changes. *Nature*, *436*(7049), 341–346. http://doi.org/10.1038/nature03874

Williams, S. T., Reid, D. G., & Littlewood, D. T. J. (2003). A molecular phylogeny of the

Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean. *Molecular Phylogenetics and Evolution*, *28*(1), 60–86. http://doi.org/10.1016/S1055-7903(03)00038-1

Wilson, N. G., Hunter, R. L., Lockhart, S. J., & Halanych, K. M. (2007). Multiple lineages and absence of panmixia in the "circumpolar" crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. *Marine Biology*, *152*(4), 895–904. http://doi.org/10.1007/s00227-007-0742-9

Wilson, N. G., Schrödl, M., & Halanych, K. M. (2009). Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology*, *18*(5), 965–984. http://doi.org/10.1111/j.1365-294X.2008.04071.x

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, *29*(22), 2869–2876. http://doi.org/10.1093/bioinformatics/btt499

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	-									

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

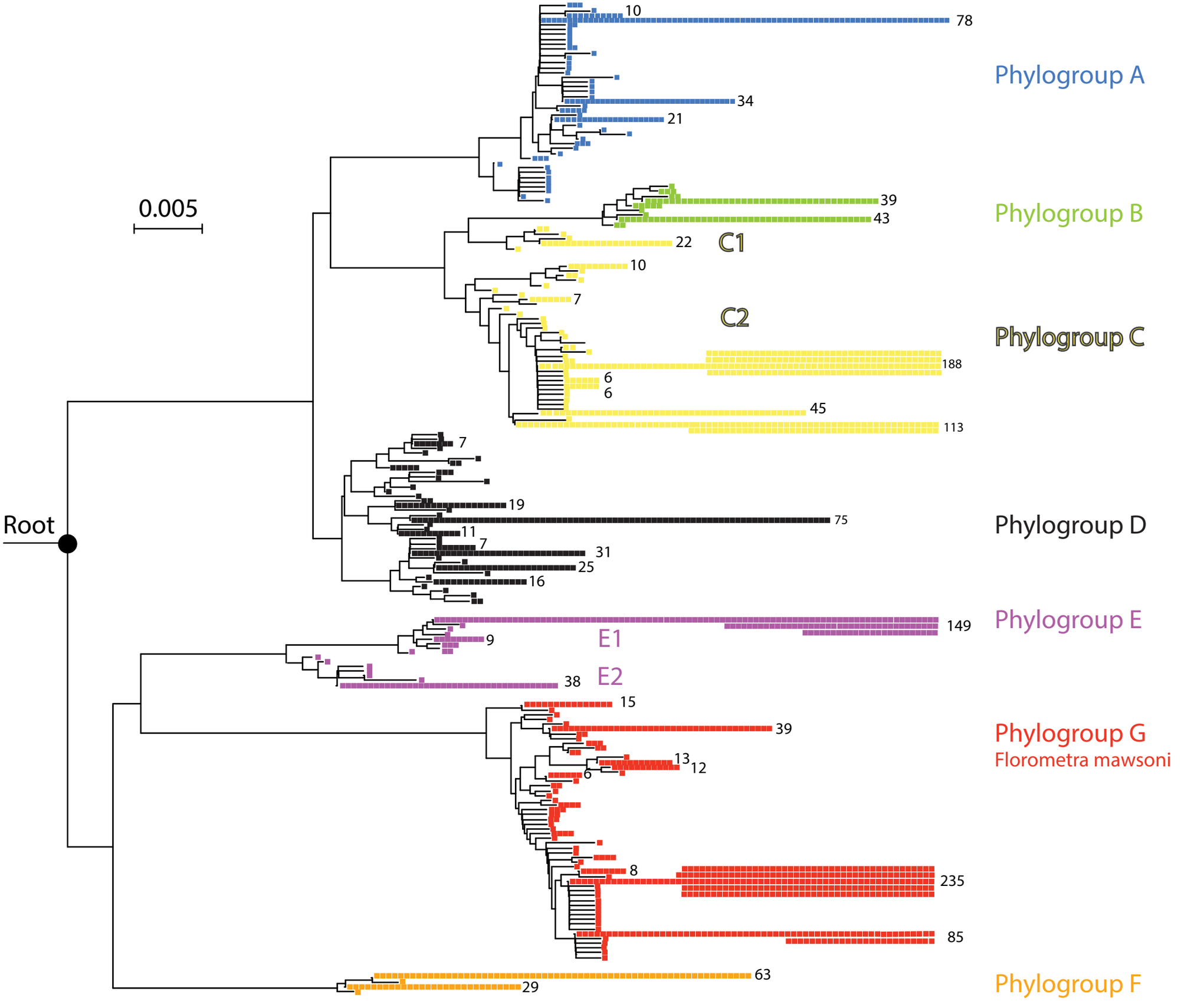
827 Summary table of effective population sizes (*Ne* in numbers of individuals) and mutation
828 rates (μ per base pair per year) for each phylogroup estimated by the Fu (2001) method. We
829 also report the years and the number sequences used for each estimation.

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	

830

831

832 **Fig. 1:** Phylogenetic representation of the 1797 COI sequences from *P. kerguelensis* and
833 *F. mawsoni*. The tree was inferred using BIONJ on the unique sequences. Each square
834 represents one sequence. The phylogroups are the ones used in the analysis (mostly ABGD
835 groups) and both supplementary splits proposed by PTP are indicated as C1/C2 and E1/E2.
836 The colors represent the different phylogroups considered in this study. The boxes and the
837 numbers refer to the number of individuals carrying the haplotype.
838
839 **Fig. 2:** Genetic turn over at the RS (a and b) and EWS (c and d) locations. The pie charts (a
840 and c) represent the number of phylogroups at different years in the locations, with the same
841 colors code as in Figure 1. The heatmaps (b and d) represents P-values of the Hudson et *al.*
842 (1992) tests for genetic structure within a phylogroup of each location.
843
844 **Fig. 3:** Distribution of p-values for different neutrality tests on the 1,624 sequences tested for
845 group with at least 10 sequences. From left to right: Tajima D (1983), Fu and Li D* (1993),
846 Fu and Li F* (1993), Achaz Y* (2008) and Strobeck S (1987).
847
848 **Fig. 4:** Simulation of migration effect on Ne estimation.
849
850 **Fig. 5:** Divergence time of the different phylogroup of the *P. kerguelensis* complex. The
851 depth of the triangles represents the maximum diversity within each phylogroup. The time is
852 indicated in years before present. The timescale on the top is assuming a mutation rate of
853 8.10^{-5} mutation/bp/year. The timescale on the bottom is assuming a mutation rate of 8.10^{-3}
854 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
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39
13
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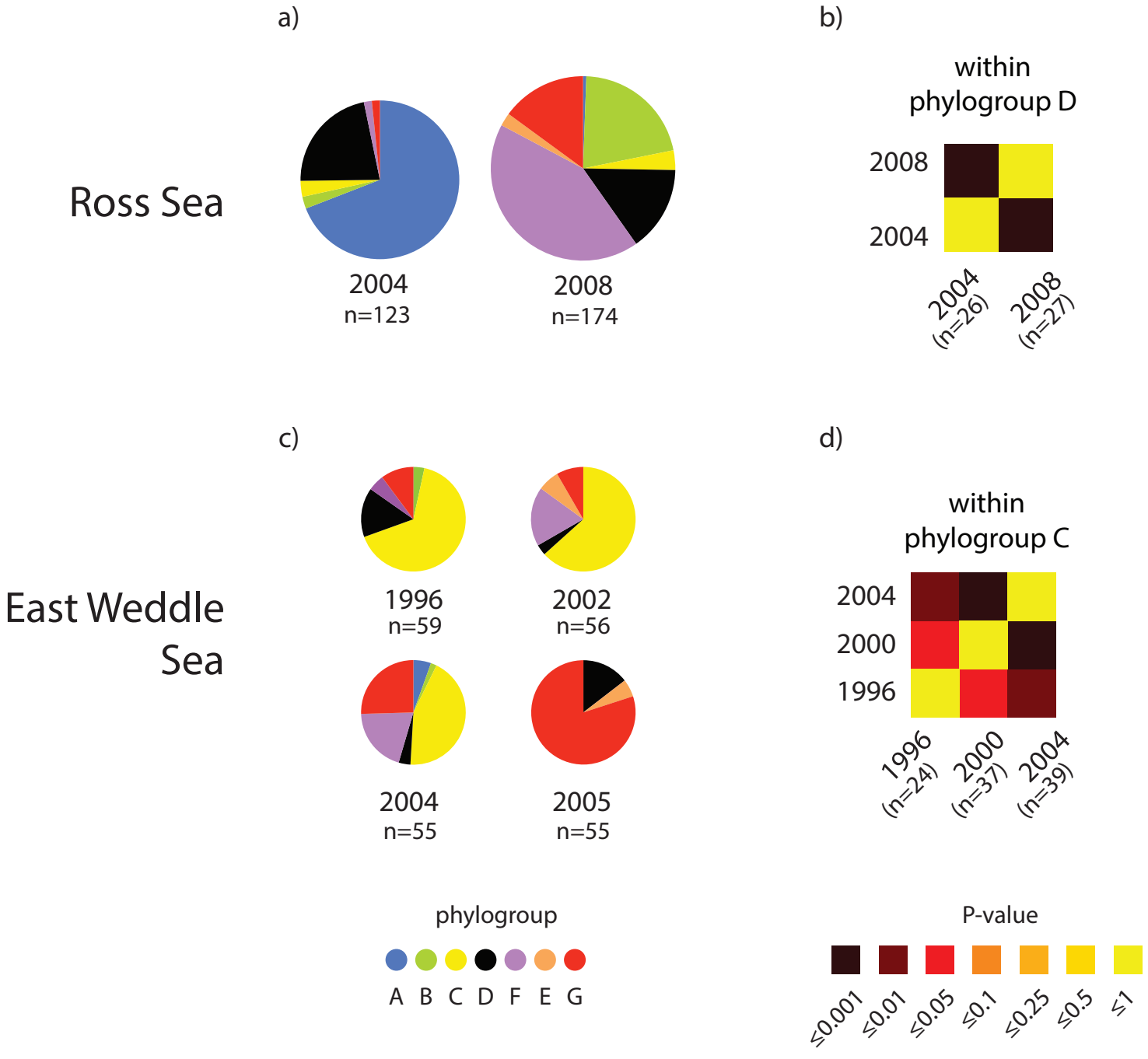
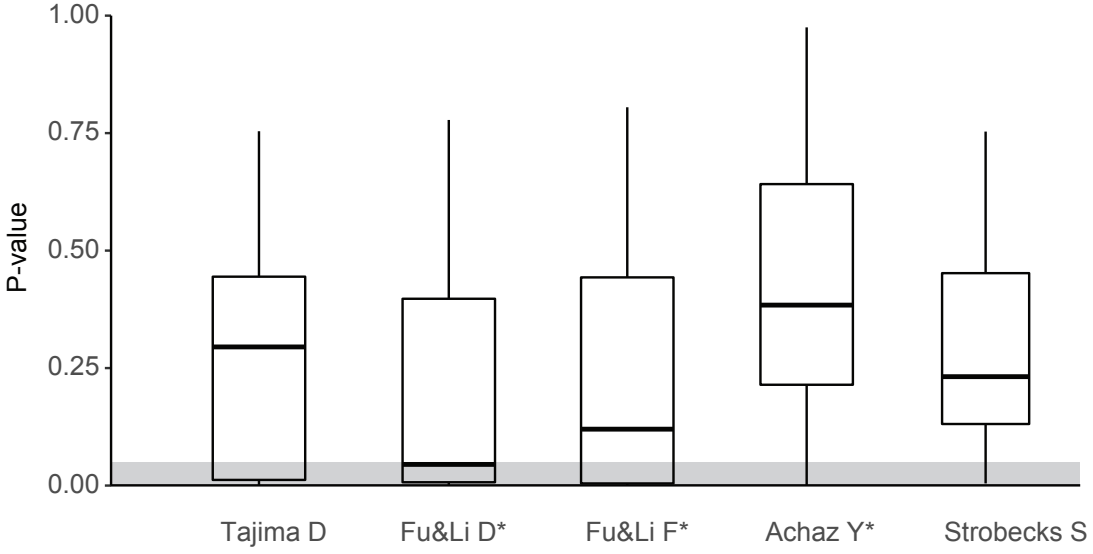
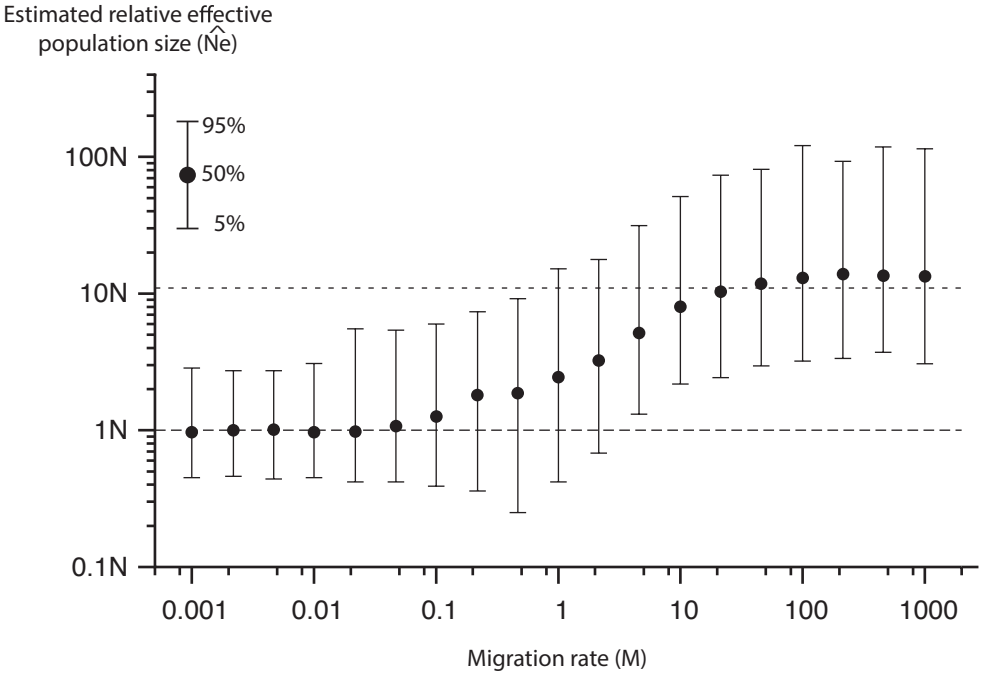
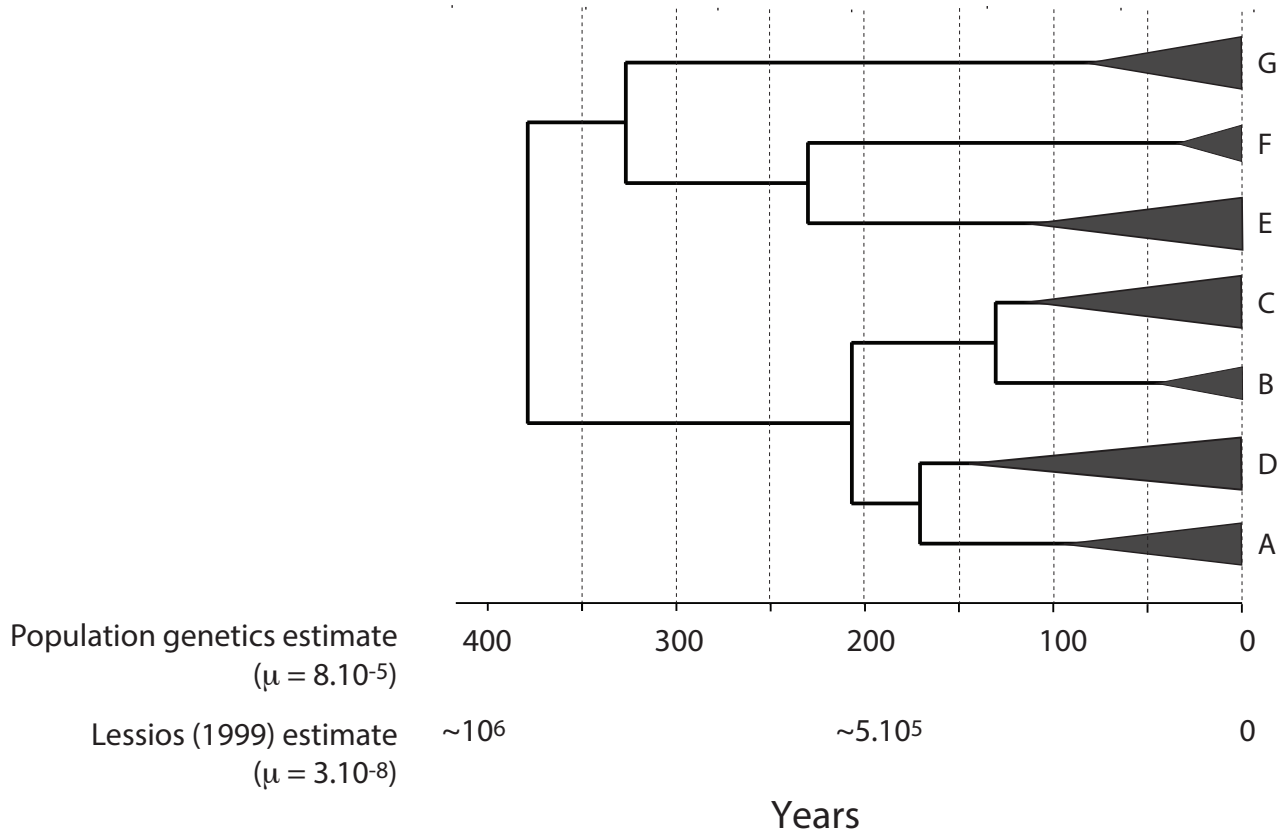
6
8
235
85
63
29


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