

Historical biogeography of the highly diverse brown seaweed Lobophora (Dictyotales, Phaeophyceae)

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1	Historical biogeography of the highly diverse brown seaweed Lobophora
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19	
20	Submission to MPE as an Original Article
21	Running title: Present and historical biogeography of Lobophora.
22	
23	Highlights
24	- Lobophora global species diversity is estimated to be >100 species.
25	- We provide a multi-locus time-calibrated molecular phylogeny for <i>Lobophora</i> .
26	- Ancestral range reconstruction suggests Lobophora originated in the Tethys
27	Sea.
28	- Lobophora molecular phylogeny revealed an upper Cretaceous origin.
29	- Within marine realms speciation and founder events were significant
30	processes.
31	
32	This research did not receive any specific grant from funding agencies in the public,
33	commercial, or not-for-profit sectors.
34	

35 ABSTRACT

36 The tropical to warm-temperate marine brown macroalgal genus Lobophora 37 (Dictyotales, Phaeophyceae) recently drew attention because of its striking regional 38 diversity. In this study we reassess Lobophora global species diversity, and species 39 distributions, and explore how historical factors have shaped current diversity 40 patterns. We applied a series of algorithmic species delineation techniques on a global 41 mitochondrial cox3 dataset of 598 specimens, resulting in an estimation of 98 to 121 42 species. This diversity by far exceeds traditional diversity estimates based on 43 morphological data. A multi-locus time-calibrated species phylogeny using a relaxed 44 molecular clock, along with DNA-confirmed species distribution data was used to 45 analyse ancestral area distributions, dispersal-vicariance-founder events, and temporal 46 patterns of diversification under different biogeographical models. The origin of 47 Lobophora was estimated in the Upper Cretaceous (-75 to -60 MY), followed by gradual diversification until present. While most speciation events were inferred 48 49 within marine realms, founder events also played a non-negligible role in Lobophora 50 diversification. The Central Indo-Pacific showed the highest species diversity as a 51 result of higher speciation events in this region. Most Lobophora species have small 52 ranges limited to marine realms. Lobophora probably originated in the Tethys Sea and 53 dispersed repeatedly in the Atlantic (including the Gulf of Mexico) and Pacific 54 Oceans. The formation of the major historical marine barriers (Terminal Tethyan 55 event, Isthmus of Panama, Benguela upwelling) did not act as important vicariance 56 events. Long-distance dispersal presumably represented an important mode of 57 speciation over evolutionary time-scales. The limited geographical ranges of most 58 Lobophora species, however, vouch for the rarity of such events.

- 59 Keywords Algorithmic species estimation; ancestral area reconstruction; historical
- 60 biogeography; *Lobophora*; molecular dating.

61 1. Introduction

62 A good understanding of species diversity is essential for addressing biogeographical 63 questions. Recent studies addressing the magnitude of eukaryotic diversity of 64 terrestrial and marine systems (Appeltans et al., 2012; Costello et al., 2013; Mora et 65 al., 2011; Scheffers et al., 2012; Sweetlove, 2011) have highlighted the large 66 uncertainty in global species diversity, with global species diversity estimates ranging 67 between 2 and 50 million species. In addition, the application of DNA markers to 68 delineate species (DNA taxonomy (DNA taxonomy; Blaxter, 2004) disclosed levels 69 of cryptic species diversity, which in many cases outnumber traditionally recognized 70 species by a factor 10 (Adams et al., 2014). Failure to recognize cryptic species not 71 only results in underestimation of species diversity, but may also have significant 72 consequences for the interpretation of macroevolutionary patterns and for species 73 conservation (Agapow et al., 2004; Bickford et al., 2007). Recent estimates of global 74 biodiversity, however, did not take into account the magnitude of cryptic species (but 75 see Appeltans et al., 2012), which is likely to be common in many organismal groups 76 (Adams et al., 2014; Pfenninger and Schwenk, 2007). Algae represents a group for 77 which the magnitude of diversity remains highly uncertain (De Clerck et al., 2013; 78 Guiry, 2012). Several regional case studies demonstrated that algal species diversity 79 could be up-scaled with one or two orders of magnitude (e.g. Evans et al., 2007; 80 Leliaert et al., 2014; Payo et al., 2013; Saunders, 2008; Stiller and Waaland, 1993), 81 recognizing the high level of cryptic species in this group (but several studies did not 82 unveil a lot of cryptic species).

Next to the uncertainty on diversity estimates, it is unclear how local diversity
estimates translate into global diversity. Estimations of the global diversity of any
given group require insights in the geographical structuring of species diversity. High

local diversity may not necessarily translate into high global diversity, because of
broad geographic ranges and/or the paucity of species diversity in other regions.
Conversely, narrow species ranges may result in relatively low species diversity at a
local scale, but high global diversity.

90 While in general diversity is lower in the sea than on land, marine species diversity in 91 some groups and areas can be high (Appeltans et al., 2012; De Vargas et al., 2015; 92 Grosberg et al., 2012; May and Godfrey, 1994; Vermeij and Grosberg, 2010). This 93 high species diversity in the marine environment raises evolutionary questions related 94 to the drivers and mechanisms of evolutionary diversification. Geographic isolation is 95 the traditional explanation for diversification, but there is growing consensus that 96 sympatric adaptive diversification may be an important source of diversity in the 97 marine environment (Bowen et al., 2013; Schluter, 1996, 2001). Opportunities for 98 allopatric speciation are reduced in the ocean since there are few physical barriers, 99 and dispersal may be extensive (Bowen et al., 2013). Although certainly true for 100 many fishes and invertebrates with pelagic larval stages that have high dispersal 101 potential (Kinlan and Gaines, 2003), long-distance dispersal is rarer in marine 102 macroalgae as propagules have been shown to have limited dispersal capabilities 103 (Kinlan and Gaines, 2003; Norton, 1992; Santelices, 1990). There are, however, 104 exceptions of macroalgal species with high dispersal capacity and wide geographical 105 ranges, including Macrocystis pyrifera (Macaya and Zuccarello, 2010), Boodlea 106 (Leliaert et al., 2009), Colpomenia (Lee et al., 2014; Lee et al., 2013), Ulva 107 (Kirkendale et al., 2013), Adenocystis utricularis and Bostrychia intricata (Fraser et 108 al., 2013). The relative scarcity of cosmopolitan marine macroalgal species, 109 confirmed by molecular methods, is evidence that long-distance dispersal is not as 110 common as in other groups (e.g. with pelagic larval stages). Indeed, many alleged 111 cosmopolitan species have eventually been shown to represent a complex of 112 genetically distinct species with more restricted distributions (De Clerck et al., 2005; 113 Leliaert et al., 2009; Tronholm et al., 2012; Zuccarello and West, 2003). The strength 114 and spatial extent of gene flow is expected to be an important determinant of the 115 spatial scale at which genetic divergence and speciation can occur (Kisel and 116 Barraclough, 2010). Studies of marine tropical fauna (mostly fishes) have highlighted 117 the possible imporance of sympatric, ecological speciation in generating diversity 118 (Bowen et al., 2013; Rocha et al., 2005). This could also hold true for marine 119 macroalgae, but speciation modes are only rarely addressed for tropical seaweeds.

120 In the present study we assess species diversity and distributions on a global scale 121 focusing on the brown macroalga Lobophora (Dictyotales, Phaeophyceae). 122 Lobophora is a pan-tropico-temperate genus that has been previously documented in 123 the Atlantic (including he Gulf of Mexico), Indian and Pacific Oceans, across both 124 hemispheres (Vieira et al., 2016) (Fig.1; this study; Guiry and Guiry, 2015). Before 125 molecular data were available, virtually all specimens, regardless of their origin, had 126 been assigned to L. variegata (J.V.Lamour.) Womersley ex E.C.Oliveira, a species 127 that is now known to be restricted to the Caribbean (Vieira et al., 2016). Recent 128 molecular studies revealed that the biodiversity of this genus has been severely 129 underestimated (Schultz et al., 2015; Sun et al., 2012; Vieira et al., 2016; Vieira et al., 130 2014). This exceptional diversity discovered from limited locations in the Pacific and 131 Atlantic suggests the existence of a much greater diversity on a global level.

The present study aims to (1) assess species diversity on a global scale using molecular data, (2) define current species distributional ranges, (3) determine the role of dispersal barriers, and (4) examine spatial and temporal patterns of diversification and dispersal of the genus *Lobophora*. 136

137 **2. Material and methods**

138 **2.1. Taxon sampling**

Taxon sampling consisted of 598 *Lobophora* specimens. Sampling was carried out from the intertidal down to 90 m deep by scuba diving, snorkeling, or box-dredging (e.g. Gulf of Mexico). Specimens were sampled in more than 40 countries, spanning the entire range of the genus (Fig. 1, Vieira et al., 2016 appendix). Voucher specimens were preserved in silica gel and mounted on herbarium sheets. Collection information and voucher/herbarium numbers are detailed in Vieira et al. (2016).

145 **2.2. DNA extraction, amplification and sequencing**

146 Total genomic DNA was extracted from tissue samples dried in silica gel, or in some 147 cases from herbarium specimens, using a cetyl-trimethyl ammonium bromide 148 (CTAB) extraction method following De Clerck et al. (2006) or using a DNeasy Plant mini Kit (Qiagen, Hilden, Germany). Sequences were generated from the 149 150 mitochondrial encoded cytochrome c oxidase III gene (cox3), the chloroplast encoded 151 ribulose-1,5-biphosphate carboxylase (rbcL) and the photosystem II protein D1 152 (psbA) genes. The datasets were complemented with sequences from GenBank (cf. 153 Vieira et al., 2016 appendix). Sequences were aligned using MUSCLE (Edgar, 2004) 154 in implemented eBioX 1.6 beta (Lagercrantz, 2008; available at: 155 http://www.ebioinformatics.org).

156 **2.3. Species delimitation**

Since traditional morphology-based species delimitation often yields inaccurate estimates of seaweed diversity (Leliaert et al., 2014), we defined species exclusively based on DNA sequence data. We applied different species delimitation methods i.e., the Maximum Likelihood implementation of the GMYC model (Pons et al., 2006; 161 Reid and Carstens, 2012), the Automatic Barcode Gap Discovery (ABGD; Puillandre 162 et al., 2012) and the Poisson Tree Processes model (PTP; Zhang et al., 2013) on the 163 cox3 dataset. The use of cox3 alone is enough for delineating species in Lobophora 164 (Vieira et al., 2014). GMYC and ABGD approaches were previously applied to define 165 Lobophora species from New Caledonia (Vieira et al., 2014) and the Western Atlantic 166 (Schultz et al., 2015). Application of the ML-GMYC on cox3 yielded highly similar 167 results (1) with other delimitation methods such as the Bayesian implementation of 168 the GMYC model and the Automatic Barcode Gap Discovery (Puillandre et al., 2012) 169 for the same marker, and (2) with analysis of the other markers, rbcL and psbA. 170 GMYC analyses under a single-threshold were conducted in R (R Core Team, 2014) 171 using the package "Splits" (Fujisawa and Barraclough, 2014; Monaghan et al., 2009). 172 The cox3 ultrametric tree, used to conduct the GMYC species delineation, was 173 constructed using Bayesian analyses in BEAST v1.8.2 (Drummond et al., 2012). A 174 GTR + I + Γ substitution model was identified as the best-fitting model for *cox3*, 175 based on the Akaike Information Criterion (AIC) using jModelTest 2 (Darriba et al., 176 2012). BEAST analyses were run under a relaxed molecular clock in combination 177 with a Yule tree prior. Other priors were set to default. In order to check for 178 convergence of the MCMC chains, we performed two independent runs for 10^7 generations each, starting from random trees and sampling every 10^4 generations. 179 180 MCMC output files of the independent runs were inspected in Tracer v1.6 (Rambaut 181 et al., 2014) for acceptable effective sample sizes (ESS > 200). A burn-in of 25% was 182 applied once log-likelihood values had stabilized. Maximum clade credibility trees 183 and posterior probability for the nodes were calculated using the postburnin trees 184 using TreeAnnotator 1.8.2 (included in the BEAST package).

185 **2.4. Geographical scales**

186 Different hierarchical geographical scales were considered to assess the patterns of 187 diversity and historical biogeography analyses: (1) two basins: Atlantic and Indo-188 Pacific; (2) three regions: Indo-Pacific, East Pacific and Atlantic; (3) five sub-regions: 189 Indo-Australian Archipelago (IAA; 'A' in Figs.5, 6), West Indo-Pacific ('B' in Figs.5, 190 6), Central Pacific ('C' in Figs.5, 6), East Pacific ('D' in Figs.5, 6) and Atlantic ('E' 191 in Figs.5, 6); and (4) 9 realms based on the Marine Ecoregions of the World from 192 Spalding et al. (2007): Temperate Northern Pacific, Central Indo-Pacific, Western 193 Indo-Pacific, Eastern Indo-Pacific, Tropical Eastern Pacific, Tropical Atlantic, 194 Temperate Northern Atlantic, Temperate Southern Africa and Temperate Australasia; 195 and also two climate zones: tropical and temperate.

196 **2.5. Species richness estimation and patterns of diversity**

197 Global species diversity was estimated using non-parametric richness estimators and 198 extrapolation of the rarefaction curve (Shen et al., 2003). We used sample-based 199 rarefaction, rescaled to number of individuals, to interpolate species richness per 200 individual sampled, based on the analytical formulas of Colwell et al. (2004). 201 Additionally, we computed three species richness estimators: the incidence-based 202 coverage estimator (ICE; Chao and Lee, 1992), the Chao 2 richness estimators (Chao 203 2; Chao, 1987), and the first-order Jackknife richness estimator (Jack 1; Burnham and 204 Overton, 1979). ICE distinguishes between frequent and infrequent species in 205 analysis. Jack 1 does not differentiate the species frequency and relies on the number 206 of species only found once. Chao 2 relies on the number of unique units and 207 duplicates. Extrapolation of the rarefaction curve and species richness estimators were 208 computed with the software ESTIMATES (Version 9; Colwell, 2013). We compared the 209 observed and Chao 2 estimated species diversity between the marine sub-regions in 210 order to compare the level of diversity in each of these regions. We compared the

observed and Chao 2 estimated species diversity between four spatial scales i.e. local, sub-regional, regional and global. We took the most well-sampled locality (New Caledonia), realm (Central Indo-Pacific) and region (Indo-Pacific), in order to get the best idea of what it takes in terms of sampling to properly assess species diversity at a given spatial scale. Finally, to evaluate species range overlap between marine realms, we calculated the similarity matrix between the nine marine realms with respect to their species overlap, applying the Sørensen index (Magurran, 2013).

218 **2.6. Reconstruction of species phylogeny**

219 Based on the results of the species delimitation analyses, a concatenated alignment of 220 the cox3 (610 bp) + psbA (919 bp) + rbcL (1.360 bp) dataset was made containing a 221 single representative per species. The matrix was 80% filled at the species \times gene 222 level. Species used as outgroup taxa used for the time-calibrated phylogeny are given 223 in Table S1. Maximum Likelihood (ML) and Bayesian Inference (BI) species trees 224 were generated from the concatenated alignment, partitioned by gene and codon 225 position. ML analyses were conducted using RAxML under a GTR+CAT model 226 (Stamatakis, 2006). The robustness of the resulting phylogenies was tested using 227 1,000 replicates of a rapid bootstrap heuristic (Stamatakis, 2006); and for the BI, 228 using MrBayes v3.2.2 (Ronquist and Huelsenbeck, 2003), initiated with a random 229 starting tree and with four chains of MCMC iterations ran simultaneously for 100 230 million generations. The first 100,000 (25%) trees sampled were discarded as burn-in, 231 based on the stationarity of lnL for all parameters as assessed using Tracer version 1.6 232 (Rambaut et al., 2014). A consensus topology and posterior probabilities of the nodes 233 were calculated from the remaining trees.

234 **2.7. Time-calibrated species phylogeny**

235 The occurrence of Phaeophyceae as fossils is rare due to their generally soft-bodied 236 nature (Arnold, 1947), and scientists continue to debate the identification of some 237 fossils (Coyer et al., 2001). Padina and Newhousia are the only two genera of the 238 class Phaeophyceae which deposit calcium carbonate. While no fossils of Newhousia 239 are documented to date, the Early Cretaceous (-145.5 to -99.6 Ma) clay shales from 240 the Gangapur formation (Andhra Pradesh state, India) yielded a macroalgal fossil 241 reminiscent of extant species of the genus Padina (Rajanikanth, 1989). Babcock et al. 242 (2012) reported a new species of Padina from the Drumian Stage (Cambrian) in 243 Hunan, China. From our own observations of the available pictures, their 244 identification of a Padina is doubtful, and we decided not to consider this fossil since 245 it is challenging the current view of the time-scale of brown algal evolution (Brown & 246 Sorhannus, 2010). Our Lobophora phylogeny was therefore calibrated with (1) a 247 fossil of Padina, (2) the Dictyotales node as estimated in Silberfeld et al. (2010), and 248 (3) the Phaeophyceae node as estimated in Brown and Sorhannus (2010). The age of 249 Padina was constrained at -95 Ma and tailing off according to a gamma distribution 250 with shape = 3.0 and scale = 5.5 (Silberfeld et al., 2014). The split between the 251 Dictyotales and the outgroup Syringoderma, i.e. the crown group Dictyotales-252 Syringoderma, was constrained between -130 and -195 Ma using a uniform prior 253 (Silberfeld et al., 2014). The age of the split between Phaeophyceae and 254 Schizocladiophyceae lineages, i.e. the crown group Phaeophyceae-255 Schizocladiophyceae, was constrained in the Lower Jurassic between -125 and -253 256 Ma using a uniform prior (Brown and Sorhannus, 2010). The time-calibrated 257 Lobophora phylogeny (i.e. chronogram) was inferred using Bayesian analyses in 258 BEAST 1.8.2 (Drummond et al., 2012), for the concatenated (cox3 + rbcL + psbA)259 alignment partitioned by gene and codon position, using a lognormal relaxed 260 molecular clock method, with autocorrelated rates in combination with a Yule model 261 tree prior, and the GTR + I + Γ substitution model for the three unlinked markers. 262 The GTR + I + Γ substitution model was identified as the best-fitting model for each 263 gene, based on the Akaike Information Criterion (AIC) using jModelTest 2 (Darriba 264 et al., 2012). Other priors were set to default. In order to check for convergence of the MCMC chains, we performed two independent runs for 10^7 generations each, starting 265 from random trees and sampling every 10^4 generations. MCMC output files of the 266 267 independent runs were inspected in Tracer v1.6 (Miller et al., 2010) for acceptable 268 effective sample sizes (ESS > 200). A burn-in was applied once log-likelihood values 269 for all parameters had stabilized. Maximum clade credibility trees and posterior 270 probabilities for the nodes were calculated using the postburnin trees using 271 TreeAnnotator 1.8.2 (included in the BEAST package). All phylogenetic analyses 272 were conducted on the Cipres web portal (Miller et al., 2010).

273 2.8. Historical biogeography

To infer the evolution of geographical ranges, we used the R package BIOGEOBEARS (Matzke, 2013). This package implements the most common biogeographical history reconstruction methods in a likelihood framework: dispersal-extinction-cladogenesis model (DEC; Ree et al., 2005; Ree and Smith, 2008), dispersal-vicariance analysis (DIVA; Ronquist, 1997) and the BayArea model (Landis et al., 2013). Moreover, it also incorporates a model of founder-event speciation ('+J') and allows the fit of models to be compared using a model choice procedure (Matzke, 2013).

281

282 **3. Results**

283 **3.1.** *Lobophora* global species diversity

284 The GMYC analysis based on the mitochondrial cox3 marker, significantly rejected 285 the null model (single coalescence model for the entire tree), resulting in delimitation of 109 evolutionary significant units (ESUs) (Fig. 2), with a confidence interval of 98 286 287 - 121. ABGD and PTP resulted in the delineation of 100 and 141 ESUs, 288 respectiveley. ABGD lineages were subdivided 5 and 26 times by GMYC and PTP, 289 respectively. Subdivisions of GMYC and ABGD lineages in the PTP delineation, 290 generally reflected the biogeography of the lineages, i.e. sibling lineages in PTP are 291 geographically distinct. For example L. sp.35 split into two lineages in the PTP 292 analysis, which correspond to two regions, namely South Africa and Juan de Nova. 293 For this study, we considered the GMYC delineation results since the geographic 294 distances between these additional species delineated by PTP generally did not extend 295 further than within a marine region.

Extrapolation of the rarefaction curve indicates a mean value of ~190 *Lobophora* species, with a confidence interval of 140 – 235 species (Fig. S1). The species diversity value reaches a plateau at ca. 3000 samples. Species richness estimators projected a diversity of 179 (Jack 1) to 209 (ICE) species (Table1). Taking the mean and the confidence interval of the GMYC results into consideration, and estimators and extrapolation values, we estimate having discovered 42 to 86 % of the *Lobophora* extant species diversity (Table 1).

303

304 **3.2. Regional diversity**

Substantial differences in species diversity were observed between some marine subregions. The Indo-Pacific stands out with the highest diversity with 95 species and an
estimate of 150 species based on the Chao2 species richness estimator (Fig. 3A, S2A).
The level of diversity drops to 18 species in the Atlantic with an estimate of 20

309 species based on Chao2 (Fig. 3A, S2A). The least speciose regions are Temperate 310 Australasia and the Tropical Eastern Pacific with six and four species, respectively, 311 and with similar Chao 2 based-estimates (Fig. 3A, S2A). We also examined species 312 diversity along a multiscale gradient from a local (i.e. New Caledonia) to a global 313 scale (Fig. 3B, S2B). Fig. 3 displays the cumulative number of species observed as a 314 function of sampling effort in different marine regions (Fig. 3A, S2A) and at different 315 scales (Fig. 3B, S2B). The shape of the curves is not the same for all regions, with 316 three out of four approximately reaching an asymptote shape, implying that the Indo-317 Pacific region reserves a greater diversity yet to be explored, while the diversity of the 318 other regions was mostly revealed by our sampling.

319

320 **3.3. Inter-regional species overlap**

321 Twenty-five percent of all Lobophora species span more than one sub-region (Fig. 6). 322 With 16 species shared with neighbouring sub-regions (nine with the Western Indo-323 Pacific and seven with the Eastern Indo-Pacific) the Indo-Australian Archipelago is 324 the sub-region that shares the most species with its adjacent sub-regions (Fig. 6). The 825 tropical Eastern Pacific, which shares no species with the Eastern Indo-Pacific and 326 only one species with the Atlantic (Fig. 6), is the most 'isolated' region followed by 327 the Atlantic (Fig. 6). A Sørensen similarity matrix shows an overall low similarity 328 (<0.20) between the nine marine realms in terms of species overlap (Table S2), 329 meaning that a limited number of species span more than one realm. The highest level 330 of similarity (0.92) is observed between the Tropical Atlantic and Temperate 331 Northern Atlantic, which have four species in common. Despite the high diversity in 332 the Indo-Pacific, provinces display low species overlap.

333

334 3.4. Geographical diversity patterns

335 The Central Indo-Pacific is the richest realm with at least 57 species, followed by the 336 Western Indo-Pacific with 35 species, the Eastern Indo-Pacific with 19 species and 337 the Tropical Atlantic with 14 species. The remaining realms contain between one to 6 338 species (Table 2). Only three species are occurring across both hemispheres (L. 339 asiatica, L. sp.18 and L. sp44). Ninety-nine Lobophora species (87%) are strictly 340 tropical, 5 species (4%) are strictly temperate and 10 species (9%) are tropico-341 temperate (present in warm temperate and tropical regions). Nearly all Lobophora 342 species (109 species = 97%) are restricted to one ocean basin (Table 2), and 86 343 species (75%) are restricted to one marine realm (Table 2). Twenty-three (20%) and 344 five (3.5%) species are spanning two and three realms, respectively. In the Indo-345 Pacific, only four species are distributed across the centro-western part (L. sp28 (8 346 specimens), L. rosacea (67 specimens), L. gibbera (7 specimens), L. ceylanica (5 347 specimens)) and only three in the centro-eastern part (L. pacifica (11 specimens), L. 348 undulata (31 specimens), L. sp19 (20 specimens)), but in our dataset no species are 349 found across the entire the Indo-Pacific.

350

351 **3.5. Dated molecular phylogeny of** *Lobophora*

A *Lobophora* species tree, infered from a concatenated alignment of *rbcL*, *cox3* and *psbA* sequences, presented with maximum-likelihood bootstrap and Bayesian posterior probability values, is given in Fig. S3. Our time-calibrated phylogeny indicates that *Lobophora* originated in the Upper Cretaceous between 65 – 90 MY (Fig. 4). From the beginning of the Cenozoic onward, *Lobophora* diversification occurred rather steadily through its evolutionary history (Fig. 4). None of the major marine vicariance events (e.g. closure of the Tethys Sea, Benguela upwelling, Panama 359 Isthmus closure; indicated as vertical lines in Fig. 4) nor sea level variations 360 (indicated as a blue line in Fig. 4) seem to have caused major shifts in diversification 361 rates of *Lobophora*. On the other hand, the East Pacific barrier represents a clear 362 dispersal barrier since the East Pacific has a lower number of *Lobophora* species, and 363 only three that span the central and eastern Pacific.

364 **3.6. Historical biogeographical inference**

365 The Dispersal-Extinction-Cladogenesis with founder-event speciation model (DEC+ 366 J) was identified as the best model in the BioGeoBEARS analyses when considering 367 nine marine realms or five marine sub-regions (Table 3). These results highlight the 368 importance of founder-event speciation (j=0.0254 > d=0.0019 > e=0). When the 369 number of regions was reduced to three (Atlantic, Indo-Pacific and Eastern Pacific) or 370 two (Atlantic and Indo-Pacific), DIVA + J was identified as the best model. Based on 371 the historical biogeographical inference based on the basins level (Atlantic and Indo-372 Pacific), the DEC + J model informs us that the *Lobophora* ancestor (LA) originated 373 from the Indo-Pacific which corresponded to the Upper Cretaceous Tethys Sea (Figs. 374 4, 5).

375 **3.7. Relative contribution of sympatry, vicariance and founder events**

376 "Biogeographical Stochastic Mapping" (BSM), implemented in BioGeoBEARS, 377 allows to quantify speciation events. 'Sympatric' speciation, i.e. speciation within a 378 predefined region, comes as the most important speciation mode (90%) at the basin 379 level, with the remaining 10% being founder events, e.g. dispersal from one basin on 380 to another. At a finer scale, i.e. marine realms level, speciation within marine regions 381 remains the most important mode of speciation (71%), followed by founder events 382 (19%) and vicariance (9%). The relative contribution of each of these modes of 383 speciation varies between the different realms (Figs. 6,S5). For instance, while most of *Lobophora* diversity within the Central Indo-Pacific and the Western Indo-Pacific
result from 'sympatric' speciation, *Lobophora* diversity within the Temperate
Northern Pacific and Temperate Southern Africa exclusively results from founder
events (Figs. 6,S5).

388

389 **4. Discussion**

390 4.1. Species diversity

391 We assessed species diversity of the marine brown algal genus *Lobophora* on a global 392 scale. The level of Lobophora diversity unveiled from local studies in the Pacific 393 Ocean (Sun et al., 2012; Vieira et al., 2014) already predicted a richer global 394 biodiversity for this genus than previously recognized. Our DNA sequence data 395 indicate an increase of the species diversity of the genus Lobophora by five to six 396 folds, from 20-30 species to more than 100 species, which makes Lobophora a 397 hyperdiverse genus of marine macroalgae. Our results once again show how 398 morphology-based taxonomy fails to accurately estimate species diversity in some 399 algal groups (De Clerck et al., 2013; Leliaert et al., 2014; Packer et al., 2009).

400 **4.2. Geographic distributions**

401 While sister species may be geographically widely separated (Fig. S4), the 402 distribution of single species are mostly restricted to one ocean basin and usually do 403 not expand beyond marine realms as defined by Spalding et al. (2007), but there are 404 exceptions, namely L. sp37, L. sp44 and L. sp77, which are spanning beyond the 405 Atlantic. Not a single Lobophora species was found to be pantropical, i.e. 406 cosmopolitan. Several other algal taxa with allegedly broad distribution have been 407 shown to correspond to complexes of species with restricted distributions. A study 408 conducted on the genus *Padina* (Silberfeld et al., 2014) (a member of the same family

409 as Lobophora, Dictyotaceae) resolved that globally distributed morphospecies 410 segregated into evolutionary lineages with more restricted ranges. Working on two 411 supposedly circumtropical Dictyota species (also Dictyotaceae), D. ciliolata and D. 412 crenulata, Tronholm et al. (2012) concluded that the former consisted of several 413 pseudocryptic species with restricted distributions in the Atlantic Ocean and Pacific 414 Central America, while the pantropic distribution of the latter was confirmed. Other 415 examples can be given, such as *Colpomenia sinuosa* which consist of several species 416 with more or less wide distribution (Lee et al., 2013). Although red algae may have a 417 different evolutionary history, at a finer geographical scale, Zuccarello and West 418 (2003) study on the Bostrychia radicans/B. moritziana complex resulted in the 419 identification of distinct evolutionary lineages with defined areas along the eastern 420 North American coast.

421 **4.3. Patterns of diversity**

422 The majority of the *Lobophora* species are restricted to tropical regions, and have 423 small ranges limited to marine realms. Lobophora species diversity is highest in the 424 Indo-Australian Archipelago (IAA). In contrast to the general patterns of most 425 macroalgal genera (e.g. Santelices and Marquet, 1998), the center of diversity for the 426 genus Lobophora is located in the tropics. Similar patterns are observed among 427 several other macroalgal groups such as siphonous green algae (Kerswell, 2006), but 428 also genera belonging to the same order as *Lobophora*, i.e. *Dictyota* (Guiry and Guiry, 429 2015) and Padina (Silberfeld et al., 2014). In the Atlantic Ocean, the center of 430 diversity is located in the central Caribbean. However, diversity in the Atlantic (14 431 species) is much lower compared to the Indo-Pacific (102 species). Several possible 432 explanations have been discussed in the literature, e.g. greater diversity and extent of 433 shallow water habitats in the central Indo-Pacific compared to the Atlantic (both 434 historically and today); bigger size of the tropical Indo-Pacific, and higher number of 435 islands, relative to the Atlantic, providing more opportunities for isolation and 436 speciation; greater age of the Indo-Pacific; increased diversification in Oligo-Miocene 437 related to tectonic activity in central IWP (e.g. collision of Australia-New Guinea 438 plate with SE Eurasia) increasing shallow water habitats (Williams and Duda Jr, 439 2008); increased speciation by isolation and reconnection of Indo-Pacific populations 440 as sea levels drop and rise (e.g. during Pleistocene glaciation events); ecological 441 speciation (e.g. Bowen et al., 2013, see discussion below). Then, there is the classical 442 discussion whether the high diversity in the central Indo-Pacific is the result of high 443 speciation rates within the region (center of origin), speciation in peripheral regions 444 and dispersal and survival in the central IP (center of accumulation), or the result of 445 overlapping ranges (center of overlap) (Barber and Meyer, 2015).

446 **4.4. Tethyan diaspora: origin and early diversification**

447 The time-calibrated phylogeny and historical biogeographical analysis suggest that 448 Lobophora originated in the Upper Cretaceous in the remains of the Tethys Sea. 449 Origination in the Tethys Sea is inferred, yet with a high level of uncertainty, as 450 possible/putative ancestral area a region common to the current Atlantic and Indo-451 Pacific Oceans. On the other hand the ancestral range of the large clade 452 emcompassing L. sp61 as outgroup, that originated -55 My, is inferred to be the 453 Central Indo-Pacific with high certainty. From the Tethys Sea, Lobophora species 454 experienced multiple, more recent, dispersal events to the Atlantic, with little 455 diversification within the region. Diversification has been considerably higher in the 456 Indo-West Pacific compared to the Atlantic and Eastern Pacific (see discussion 457 above). Nevertheless, dispersal events in the Atlantic Ocean may have occurred more 458 recently than during the Upper Cretaceous (e.g. Oligocene and Miocene) which could 459 explain why the number of species is lower in the Atlantic. In fact, while Atlantic 460 species branched off early in the tree, dispersal events could have taken place 461 anywhere along these branches (e.g. the actual dispersal event could have taken place 462 close to the nodes of Atlantic clades). Generally, founder speciation occurred several 463 times throughout Lobophora evolutionary history thus playing an important role in its 464 diversification. High diversity within the Central Indo-Pacific region may have 465 resulted from a combination of within region speciation and of regular colonization 466 from adjacent regions (West Indo-Pacific and Eastern Indo-Pacific; Fig. 6). Furthermore, 70% of the species distributed within at least two different marine 467 468 realms are present in the Central Indo-Pacific. These observations suggest that this 469 region acted not only as a region of origination/diversification but also a center of 470 overlap (Barber, 2009; Connolly et al., 2003; Halas and Winterbottom, 2009). 471 Colonization of the Caribbean occurred several times and from different origins, and 472 resulted in very low regional diversification (e.g. Altantic clades of maximum two 473 species). The presence of only two species (L. sp44 and L. sp37) distributed in the 474 Western Indo-Pacific and in the Eastern Atlantic also suggests that while the 475 Benguela upwelling may represent an efficient dispersal barrier, dispersal across it 476 occurred at least twice. Finally, colonization of temperate regions occurred at 477 different periods of Lobophora evolution history. The earliest dispersal to temperate 478 region occurred during the Paleocene (-60 Ma) in the southern hemisphere. Northern 479 hemisphere temperate regions were colonized more recently. The current global 480 Lobophora taxonomic makeup shows that hard barrier formations (East Pacific 481 Barrier, Terminal Tethyan event, Isthmus of the Panama) did not act as important 482 vicariance events for this genus (Fig. 4). On the other hand, they constituted efficient 483 barriers for Lobophora dispersal. However, while no sibling species were yet found 484 across the Panama isthmus, it is not excluded that a more significant sampling effort485 on the Pacific side of the isthmus could disclose sibling species.

486 **4.5. Geographical speciation processes**

It is important to note that the term 'sympatric' speciation expresses here speciation within the scale considered (e.g. basin, region, realm). Within a given region, however, speciation may actually result from allopatric speciation (e.g. vicariance or founder event). Identifying actual sympatric speciation events requires working at the finest possible scale e.g. several hundred meters to several hundred kilometers.

492 We analyzed diversification at different scales from ocean basins to the marine 493 realms. Virtually all speciation events occurred within ocean basins, and two-third of 494 the speciation events occurred within marine realms. On the other hand, long-distance 495 dispersal to adjacent realms, followed by founder speciation represents a non-496 negligible process in Lobophora diversification. It is difficult, however, to make 497 sound conclusions regarding geographical versus ecological speciation modes based 498 on our data. Within realms, the finest scales considered in this study, sympatric and/or 499 allopatric speciation could have occurred. Finer phylogeographic studies, down to 500 scales of several kilometers, in combination with ecological data, will be needed to 501 assess the relative role of allopatric versus sympatric speciation in Lobophora (e.g. 502 Billard et al., 2010; Payo et al., 2013; Pielou, 1978). The wide ecological variation in 503 Lobophora hints toward an important role of ecological speciation. In addition, 504 ecological partitioning has been shown to allow coexistence of sympatric Lobophora 505 sister species (Vieira et al., 2014) often found only several meters apart.

506 **4.6. Cladogenic drivers**

507 *Lobophora* species distribution and richness are reminiscent of those of corals and508 coral reef fishes (Cowman and Bellwood, 2011). Several studies have already pointed

509 to the central role of coral reef association in underpinning diversification within 510 major marine groups (Alfaro et al., 2007; Bellwood et al., 2010; Cowman and Bellwood, 2011; Hughes et al., 2002; Renema et al., 2008). Considering the major 511 512 role herbivory played in macroalgal ecology (Hay, 1997; Lubchenco and Gaines, 513 1981), diversification of reef algae and herbivores are very likely correlated through a 514 co-evolutionary arms race. The development of a complex mosaic of reef habitats also 515 probably favored reef algal speciation by providing opportunities for new habitat 516 colonization and ecological diversification (Alfaro et al., 2007; Cowman and 517 Bellwood, 2011). Thus, the biotic interactions between Lobophora, herbivores and 518 corals may have favored diversification in coral reefs. This idea that coral reefs acted 519 as cladogenesis drivers has already been proposed for other reef organisms, such as 520 coral reef fishes, where coral reefs provided the mechanisms allowing both higher 521 rates of speciation and reduced vulnerability to extinction for associated lineages 522 (Cowman and Bellwood, 2011).

523 Lobophora has been considered as a potent competitor with corals. An example is its 524 proliferation following disturbances that impacted herbivores and corals in the 525 Caribbean in the mid-80s (De Ruyter van Steveninck and Breeman, 1987; Hughes, 526 1994). Timing of origination and patterns of distribution and diversity clearly show 527 that Lobophora is a fully-fledged member of coral reefs and has evolved in these 528 ecosystems since the rise of modern coral reefs (during the Cretaceous). 529 Consequently, Lobophora should not be seen as a threat to corals, but instead as an 530 indicator of coral reef health status. In fact, while following disturbances, Lobophora 531 has shown the capacity to bloom in certain reefs across the globe (De Ruyter van 532 Steveninck and Breeman, 1987; Diaz-Pulido et al., 2009; Lesser and Slattery, 2011), 533 and corals demonstrated resilience once conditions came back to normal (Diaz-Pulido

- et al., 2009). In healthy reefs, *Lobophora* has been reported only once as representing
 an apparent threat to corals (Vieira et al., 2015), a case of epizoism syndrome, but
 even then, only one species was threatened.
- 537

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 reproductive isolation in the *Bostrychia radicans/B. moritziana* complex
 (Rhodomelaceae, Rhodophyta) with focus on North American isolates. J.
 Phycol. 39, 948-959.

816

817 818 Table 1. Number of estimated species and resultant percentage of species discovered. The number of species is

estimated with the species-richness estimators (ICE, Chao 2 and Jack 1) and with the extrapolation (mean and 819 lower and upper 95% confidence interval). The percentage of species discovered based on the number of estimated

820	species and	l the numbe	er of discovered	l species identified.	
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	Richness estimators			Extrapolation				
	ICE Chao 2 Jack 1			Lower 95%	Mean Unner 959			
No. of species ⁽¹⁾	209	185	179	140	188	235		
Low DS (%) ⁽²⁾	47	53	55	70	52	42		
Mean DS (%) ⁽³⁾	52	59	61	78	58	46		
Upper DS (%) ⁽³⁾	58	65	68	86	64	51		

⁽¹⁾Number of estimated species. Percentage of discovered species considering the mean and the lower (⁽²⁾98) and upper (⁽⁴⁾121) 95% confidence interval number of species identified with the GMYC model based on cox3. DS: described species.

824 Table 2. Lobophora species diversity per marine region.

	Species # (%)
Tropical	109 (81)
Temperate	15 (11)
Tropico-temperate	10 (7)
Pacific	102 (87)
Atlantic	15 (10)
Pacific-Atlantic	4 (3)
Indo-Australian Archipelago	60 (39)
Western Indo-Pacific	36 (23)
Central Pacific	19 (12)
Eastern Pacific	4 (3)
Atlantic	15 (10)
Central Indo-Pacific	57 (31)
Western Indo-Pacific	35 (19)
Eastern Indo-Pacific	19 (10)
Temperate Australasia	6 (3)
Temperate Northern Pacific	2 (1)
Tropical Eastern Pacific	4 (2)
Temperate Southern Africa	1 (1)
Tropical Atlantic	14 (8)
Temperate Northern Atlantic	7 (4)

825 826 827 828 829 830 831 832 Table 3. Comparison of the fit of the dispersal-extinction-cladogenesis (DEC), dispersal-vicariance analysis (DIVA) and BayArea biogeographical reconstruction models, all with the possibility of founder-event speciation ('+J'). The log-likelihood (lnL) of each model is given for the analyses. For each geographical subdivision, the best model is indicated in bold. ¹9 realms: Central Indo-Pacific, Western Indo-Pacific, Eastern Indo-Pacific, Temperate Australasia, Temperate Northern Pacific, Tropical Eastern Pacific, Temperate Southern Africa, Tropical Atlantic, Temperate Northern Atlantic.² 5 regions: Indo-Australian Archipelago, Western Indo-Pacific, Central Pacific, Eastern Pacific, Atlantic. ³ 3 regions: Atlantic, Pacific, Indian Ocean. ⁴ 2 basins: Atlantic, Indo-Pacific.

	9 realms ¹	5 regions ²	3 regions ³	2 basins ⁴	Temp-Trop
DEC	-316.5	-248.0	-69.8	-50.2	-46.3
DEC+J	-298.1	-219.9	-63.3	-46.4	-46.3
DIVA Like	-324.8	-248.3	-64.4	-46.7	-51.1
DIVA Like + J	-309.1	-226.8	-62.6	-45.9	-51.1
BayArea Like	-339.9	-280.6	-97.9	-72.8	-53.5
BayArea Like + J	-313.8	-231.3	-66.6	-50.1	-53.3

833

834 835 Table S1. Outgroups used to root the Lobophora tree brown algal tree with their GenBank accession number.

Species cox3 rbcL	psbA
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⁸²¹ 822 823

Canistrocarpus cervicornis	LN871906	-	LN831806
Choristocarpus tenellus	-	AB899285	AB899261
Cladostephus spongiosus	-	FN667651	-
Cutleria multifida	LC074884	AB776782	AB543588
Desmarestia ligulata	EU681444	AJ287848	EU681637
Discosporangium mesarthrocarpum	-	AB252654	AB899262
Ectocarpus siliculosus	AB526435	AY307410	FP102296
Fucus vesiculosus	AY494079	DQ307680	DQ307679
Ishige okamurae	FJ427586	AY372975	AY528830
Laminaria digitata	AJ344328	AY372984	AY528849
Phaeothamnion confervicola	-	AF064746	HQ710732
Schizocladia schiensis	-	AB085615	AY528859
Sphacelaria divaricata	-	AJ287889	AY528855
Syringoderma phinneyi	EU681467	AJ287868	AY528858
Undaria pinnatifida	GQ368282	GQ368325	GQ368354

837 Table S2. Similarity matrix of *Lobophora* the species composition in 9 marine realms calculated with the Sørensen index.

	CIP	WIP	EIP	Tau	TNP	TEP	TSA	TAtl	TNA
Central Indo-Pacific (CIP)	1	-	-	-	-	-	-	-	-
Western Indo-Pacific (WIP)	0.20	1	-	-	-	-	-	-	-
Eastern Indo-Pacific (EIP)	0.16	0.04	1	-	-	-	-	-	-
Temperate Australasia (TAu)	0.13	0.05	0.08	1	-	-	-	-	-
Temperate Northern Pacific (TNP)	0.03	0.00	0.10	0.00	1	-	-	-	-
Tropical Eastern Pacific (TEP)	0.00	0.00	0.00	0.00	0.33	1	-	-	-
Temperate Southern Africa (TSA)	0.00	0.06	0.00	0.00	0.00	0.00	1	-	-
Tropical Atlantic (TAtl)	0.03	0.04	0.06	0.00	0.00	0.2	0.00	1	-
Temperate Northern Atlantic (TNA)	0.03	0.05	0.08	0.00	0.00	0.18	0.25	0.92	1

840 **Figure caption**

Figure 1 (A) *Lobophora* global distribution range based on DNA confirmed samples
(black circles) and literature records (white circles). Pictures of (B) *L. undulata*thallus, (C) *L. rosacea* growing at the basis of branching *Acropora* corals in New
Caledonia, (D) *L. obscura* growing on dead corals in New Caledonia, and (E) *L. canariensis* growing on bedrock in the Canary Islands.

Figure 2 Results of the three species delimitation methods based on the *cox*3 data set.
Species delimitation results of GMYC (inner), ABGD (middle), and PTP (outer) are
represented by three concentric circles. The tree is the maximum clade credibility tree
obtained from BEAST. Only the terminal part of the tree, used for species delineation,
is represented. In the center, a Venn diagram illustrating overlap (consensus) between
the PTP, GMYC and ABGD species delimitation results.

Figure 3 Observed richness (S_{obs}) in *Lobophora* species. (A) Comparison between
four marine regions: Indo-Pacific (square), Atlantic (triangle), Temperate Australasia
(circle), Tropical Eastern Pacific (diamond). (B) Comparison between multiple spatial
scales: local (New Caledonia, diamond), sub-regional (Central Indo-Pacific, circle),
regional (Indo-Pacific, triangle), and global (square).

Figure 4 Chronogram resulting from the Bayesian relaxed clock analysis with BEAST 1.8.2. The purple bars display the 95% HDP (highest probability density). Lineage-through-time (LTT) plot of *Lobophora* based on the chronogram presented in Fig. 4, with the 95% confidence intervals. The red vertical lines display the emergence of major marine barriers: Terminal Tethyan event (ca. -18 Ma), the Isthmus of Panama (ca. 3 Ma), Benguela upwelling formation (ca. 1-2 Ma). The blue line displays sea-level variation based on Miller et al. (2005). 864 Figure 5 Ancestral ranges of *Lobophora* species, estimated under a DEC+J model 865 with BioGeoBears based on the BEAST tree (Fig. 4). Boxes at the tips indicate extant 866 Lobophora species geographic area(s). Colored branches indicate regions of maximal 867 probability. Ancestral area reconstructions are shown by pie diagrams at each node. 868 World map (inset lower left) shows the 5 marine realms: Central Indo-Pacific (A), 869 Western Indo-Pacific (B), Eastern Indo-Pacific (C), Tropical Eastern Pacific (D), 870 Atlantic (E). World map (inset middle left) depicts Late Cretaceous continental 871 configuration with the hypothetical center of origin of *Lobophora* (ABE).

872 Figure 6 Sympatry and founder events across the five marine realms. The five marine 873 realms are represented by anotated colored circles: Central Indo-Pacific (A), Western 874 Indo-Pacific (B), Eastern Indo-Pacific (C), Tropical Eastern Pacific (D), Atlantic (E). 875 Numbers within squircles indicate the total number of species per realm. Numbers 876 adjacent to the curved arrows indicate the number of founder events from one realm 877 to another. Numbers within circle arrows indicate the number of sympatric events 878 within a realm. Numbers at the intersection between circles represent the number of 879 species shared between these two realms.

880

Figure S1 *Lobophora* species richness estimation by extrapolation of the rarefaction curve with 95% confidence interval. Continuous black line represents the observed species richness and the black dashed line represents the estimated diversity by extrapolation. The grayed out area represents the 95% confidence interval.

885

Figure S2 Estimated richness based on the non-parametric richness estimator Chao 2
in *Lobophora* species. A. Comparison between four marine regions: Indo-Pacific
(square), Atlantic (triangle), Temperate Australasia (circle), Tropical Eastern Pacific

(diamond). B. Comparison between multiple spatial scales: local (New Caledonia,
diamond), sub-regional (Central Indo-Pacific, circle), regional (Indo-Pacific, triangle),
and global (square).

892

893 Figure S3 Lobophora species tree. The tree is the maximum clade credibility tree 894 obtained from a BEAST analysis of the concatenated alignment of three genes (rbcL, 895 cox3 and psbA). The colored hemispheres shown at each node represent Bayesian 896 posterior probabilities (left part of the circle) and ML bootstrap values (right part of 897 the circle) respectively. High support (posterior probabilities >0.95 and bootstrap 898 values >0.9) is indicated in black, while low support (posterior probabilities <0.95899 and bootstrap values <0.9) is indicated in gray. No color indicates configuration 900 incongruence between the Bayesian and the maximum likelihood trees.

Figure S4 The geographic locations of sister species belonging to the *Lobophora obscura* complex (each branch was identified as a unique species). The phylogenetic
relationships between the species are given by the phylogenetic tree resulting from the
Bayesian Inference analysis.

905 Figure S5 Relative contribution of vicariance, sympatry and founder events to
906 *Lobophora* diversity at the marine realms level. The number of species per region are
907 given above each bar.

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