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High connectivity among Vesicomyid bivalves from cold seeps and deep-sea fans of Congo

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Abstract :

Chemosynthetic ecosystems are scattered in the deep ocean, harbouring highly specialized communities, among which the degree of connectivity and dispersal is scarcely studied. This gap is largely due to limited range distribution, either real or due to highly partial exploration, preventing the availability of a sufficient number of samples for population genetic analysis. For the few species that escaped this gap, large-scale panmixia was often reported, raising wonders as to the evolutionary mechanisms involved in the first steps of speciation. Vesicomyid bivalves are one of the most abundant groups of chemosynthetic fauna, for which depth was proposed as an essential driver of differentiation. Early stages of speciation are thus expected to involve genetic differentiation along depth gradients. The vesicomyid bivalve Christineconcha regab was studied across widely separated localities along the Western African margin, from the cold-seeps of Regab pockmarks located at 3150 m depth on the Congo margin to the turbiditic lobes of the Congo deep-sea fan located at 5000 m depth, using mitochondrial (COI) sequences and eight microsatellite loci. Despite rather high density in relation to high organic matter availability, results obtained showed rather low levels of genetic diversity at both mtDNA and microsatellites. The main consistent pattern of differentiation was observed across depths from Regab pockmark (South-Western part) and lobe areas (Lobe B and C). This is likely due to the largest sample sizes characterizing Regab_SW and Lobe C allowing the detection of faint genetic differentiation, and possibly to a stronger signature in the demographically declining (thus acknowledgedly under sampled) Lobe B. Other significant results were not congruent among markers, suggesting low statistical power due to limited sample size or the occurrence of chaotic genetic patchiness. Altogether, the results suggest the occurrence of effective gene flow at regional scale, and departure from equilibrium in the recently discovered lobes of the Congo River, possibly resulting from unstable environmental conditions and recurrent events of extinction recolonization.

Keywords : Deep-sea connectivity, Genetic diversity, Chemosynthetic habitats, Atlantic, Vesicomyid bivalves, Christineconcha regab, COI gene, Microsatellite markers

1. Introduction

The deep sea represents about two thirds of Earth surface, and is characterized by a diversity of habitats from the abyssal plains to seamounts, deep-sea coral reefs and chemosynthetic ecosystems. The biodiversity sheltered in those habitats still mostly remains to be characterized (Howell et al., 2021), and the dynamics of dispersal, migration and speciation among ecosystems are still poorly studied (Taylor and Roterman, 2017). Among the major chemosynthetic ecosystems, cold-seeps are known to host rich and abundant chemosynthetic communities, where reduced chemicals are used as an energy source for chemoautotrophic bacteria that function as primary producers (Paull et al., 1984; Sibuet and Olu-LeRoy, 2002). Chemosynthetic communities are highly dependent on their environment, primarily because distribution patterns of the dominant symbiont-bearing, habitat-creating taxa are linked to methane and sulfide levels and fluxes, and substrata (Sahling et al., 2002; MacDonald et al., 2003; Levin et al., 2003; Bergquist et al., 2005; Mau et al., 2006; Olu-Le Roy et al., 2007).
Since the discovery of deep-sea chemosynthetic habitats in the late 70s, one of the most puzzling issues has been the influence of past and present connectivity in the three dimensions of the Ocean on the nature of species assemblages and their geographic distribution (Corliss et al., 1979). Cold seeps have been found to share fauna with other existing chemosynthetic ecosystems, such as hydrothermal vents, whale- and wood-falls (Hecker, 1985; Smith et al., 1989; Teixeira et al., 2013). Hypotheses have arisen as to the ecological and evolutionary role and interplay of the different chemosynthetic habitats on the persistence of deep-sea communities, among which a possible function is as a stepping-stone or refugia (Shank, 2004). During the past decade along the West African Equatorial margin, a new deep-sea habitat was discovered, harbouring typical chemosynthetic fauna, not fuelled by fluid emission as hydrothermal vents and cold seeps, but rather based on reducing sediments created by organic

matter accumulation (Rabouille et al., 2009) mainly from terrestrial origin (Treignier et al., 2006). The Congo deep-sea fan represents an enormous sink of terrestrial organic matter when compared to other turbiditic systems over the world (Baudin et al., 2017), due to the Congo River, which is the second most voluminous river in the world (Milliman, 1991). This submarine canyon, connected to the Congo River followed by a deep-sea channel, is the main feature of the continental margin in the Gulf of Guinea (Babonneau et al., 2002). It cuts deeply into the shelf and continental slope, feeding a 1250 km long meandering valley that ends at the Lobes area, more than 750 km away from the coast at around 5000 m water depth (Bonnel, 2005).

These terminal lobes of the Congo deep-sea fan are -at geological scale- a unique area, in the sense that they are fuelled, due to their direct connection to the Congo River, by recurrent turbidites driven in the deep channel by high velocity currents (Khripounoff et al., 2003; Savoye et al., 2009; Vangriesheim et al., 2009). The high sedimentation rate (> 1 cm/yr) of both labile and refractory organic matter results in sediments with high carbon content (3%) and high mineralization rates (high oxygen consumption; Rabouille et al., 2009). These reducing environments thus allow the development of dense ecosystems composed of large bivalves and bacterial mats, assemblages that are otherwise rarely observed out of regions of active cold seeps (Rabouille et al., 2017). The Regab pockmark, on the other hand, is one of the cold-seep areas found in the West African continental margin, and it is located 10 km north of the Congo deep sea channel at about 3160 m water depth (Ondréas et al., 2005; Marcon et al., 2014a). It harbours in general a high community resemblance with seeps of the western Atlantic (Barbados prism, Gulf of Mexico, Blake Ridge), with abundant and diverse bivalves and tubeworms (Sibuet and Vangriesheim, 2009; Olu et al., 2010). It is also characterized for some crustaceans and bivalve species by patterns of contemporary or recent connectivity with

hydrothermal vents and cold seeps across the Atlantic Equatorial Belt (Teixeira et al., 2013). The observed megafauna communities are organized in clusters of large organisms belonging to the bivalve families Vesicomyidae and Mytilidae (Olu-Le Roy et al., 2007).

The taxonomy, phylogeny and the origins of Vesicomyid bivalves were clarified in the last decades (Decker 2012a; 2017; Johnson et al., 2017; Yang et al., 2019). One of the Vesicomyid bivalves found both at the Lobes area and the Regab pockmark is *Christineconcha regab* (Cosel and Olu 2009; Krylova and Cosel, 2011; Marcon et al., 2014b). This species is reported in few sites of the Atlantic, at depths of about 2800 to 5000 m. In the Gulf of Guinea, *C. regab* lives and is dominant on sites with high concentration of sulfides and strong seepage such as active pockmarks with cold seeps (Krylova and Cosel, 2011).

To date, genetic studies of Atlantic chemosynthetic habitats have reported a general trend of high genetic diversities and high connectivity at regional scale despite their patchy distribution (Van Der Heijden et al., 2012; Teixeira et al., 2013; Guillon et al., 2017; Yahagi et al., 2019). Seemingly, a population genetic study on the annelid tubeworm *Escarpia southwardae* from West African cold seeps has reported a high genetic diversity and a lack of genetic differentiation (Cowart et al., 2013).

Therefore, our aim was to study the genetic diversity and connectivity of *C. regab* among the newly found chemosynthetic habitat at the Congo Lobes and the Regab cold seep, with a particular focus on the potential effect of depth on the possible occurrence of genetic differentiation, a possible early step of speciation. We used mitochondrial cytochrome c oxidase subunit I (COI) and specifically developed species-specific microsatellites markers to address the connectivity between the two habitats and assess the genetic diversity and demographic history of these sites.

2. Materials and Methods

2.1. Sampling and DNA extraction

Specimens of *Christineconcha regab* were collected from the West African Equatorial margin (Figure 1, Table 1) during two oceanographic cruises WACS (Chief scientist K. Olu) and Congolobe (Chief scientist C. Rabouille). Clams were collected with the ROV Victor, mainly using nets. A few specimens embedded in sediment blade-cores in the vicinity of the aggregations targeted with nets were also included. Once on board, live specimens were either frozen entire at -20°C, or dissected under sterile conditions to condition distinct body parts either at -20°C or in 70 % ethanol. DNA extraction was performed using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1990) on muscle tissue.

Vesicomyid clams were collected from two sites: the submarine lobes of the Congo River and the Regab pockmark (Fig. 1). Within the lobes, four areas were sampled (A, B, C, F, see Figure 1). Individuals from a second cold seep site in the West African Equatorial margin, in vicinity of the Regab, was sampled (Baboon), yet due to the low sample size (only 4 specimens were recovered) only mitochondrial data were analyzed.

2.2. Microsatellite development and genotyping

To develop specific microsatellite markers for *Christineconcha regab*, total genomic DNA of three individuals from the Regab pockmark, was isolated using the CTAB method (Doyle and Doyle, 1990). A combination of an SSR-enrichment protocol with 454 pyrosequencing, was performed by a commercial company (Ecogenics GmbH, Zürich, Switzerland) to isolate the microsatellite sequences. One CT/GT enriched library was generated; the insert size of the libraries was 500-800 bp and the average read length from the

454 sequencer was 192 bp. A total of 24,574 sequences were obtained, of these 841 exhibited microsatellite repeats. A total of 209 primer pairs were designed using primer3 core (Untergasser et al., 2012). A total of 48 primer pairs were provided by the commercial company, after a first successful amplification test on three individuals. We tested all primers for polymorphism using a panel of 7 individuals. An M13-tail (TGTAAAACGACGGCCAGT) was added at the 5' end of all forward primers to enable fluorescent-dye labelling (Schuelke, 2000). From those 48 primer pairs, repeatable and reliable amplification was obtained for 8 polymorphic markers (Table S1). Sequences are available in GenBank (accession no. OP550437 through OP550441). Microsatellites and haplotypes genotypes data have been registered and are available on the repository SEANOE (https://www.seanoe.org/) under the doi: https://doi.org/10.17882/94263.

2.3. Polymerase chain reaction, sequencing and genotyping

Mitochondrial COI was amplified using the universal primers LCO1490 and HCO2198 described by Folmer et al. (1994). All polymerase chain reaction (PCR) amplifications were carried out in 50 µL volumes containing 50 ng DNA, 1X reaction buffer (GoTaq, Promega), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 0.4 U Taq DNA polymerase (GoTaq, Promega, Madison, WI, USA) and 0.6 µM of each primer. The PCR amplification was conducted on a Perkin-Elmer Gene Amp System 7200 (Waltham, MA, USA) with the following program: 2 min at 95 ºC; 35 cycles composed of 1 min denaturation at 95 ºC, 1 min at 52 ºC and 1.5 min elongation at 72 ºC, followed by a final elongation step of 7 min at 72 ºC. PCR products were purified and sequenced commercially at Macrogen, Inc. (Seoul, Korea) and GATC Biotech (Konstanz, Germany). GenBank accessions: *Christineconcha regab* COI haplotypes: OP550437 through OP550441.

Microsatellite loci were amplified by PCR, each 10 μL reaction contained 10 ng of genomic DNA, 1x Qiagen HotStart *Taq* buffer, 200 μM of dNTP's, 0.3 μM of each primer and 0.5 U of HotStart *Taq* polymerase (Qiagen). PCR amplifications were conducted on a Perkin-Elmer Gene Amp System 7200 with the following program: 15 min at 95°C; 30 cycles composed of 30 s at the annealing temperature, 30 s elongation at 72°C and 30 s of denaturation at 95ºC, followed by 1 min at the annealing temperature and a final 30 min elongation step at 72ºC. Fragments were separated on an ABI 3130 XL automatic sequencer with the internal size standard Rox 350. Alleles were scored using Peak Scanner version 1.0 (Applied Biosystems).

2.4. Data analysis

The mitochondrial dataset was analysed using ARLEQUIN version 3.5 (Excoffier and Lischer, 2010) to compute the following statistics at the sampling site level: number of haplotypes (*h*), nucleotide diversities (π_2) (Nei, 1987), and mean number of pairwise differences (π_1) (Tajima, 1983). The statistics from Fu's F_S (Fu, 1996) and Tajima's D (Tajima, 1989) were also computed, which are sensitive to departures both from selective neutrality and population size equilibrium caused by expansions or bottlenecks (Tajima, 1996; Fu, 1997). In a neutral framework, both are expected to result in negative values after a population expansion (Ray et al., 2003) or a selective sweep, whereas positive values are expected under balancing selection of recent bottlenecks.

The divergence levels among haplotypes were illustrated by building a median-joining networks were constructed with Network v. 4.1.0.9 (Bandelt et al., 1999), using the number of mutations as distance, to infer the most parsimonious branch connections between the sampled haplotypes.

For the microsatellite data, the mean number of alleles per locus (allelic diversity), the expected (H_E) and observed (H_O) proportion of heterozygotes, and the inbreeding coefficient (F_{IS}) were estimated using GENETIX 4.05 (Belkhir et al., 1994-2004). Significance levels were estimated using a permutation approach (1000 permutations). The rarefaction procedure implemented in GENCLONE (Arnaud-Haond and Belkhir, 2007) was used to calculate standardized allelic richness (A_{rich}), since the observed number of alleles in a sample is highly dependent on sample size and our samples were of unequal sizes.

The *F* estimator of genetic structure θ (Weir and Cockerham, 1984) was estimated for mitochondrial and nuclear loci and the probability of the *F*-statistics being greater than zero determined by permutation (1000 replicates) using GENETIX 4.05 (Belkhir et al., 1994-2004).

Finally, two AMOVA were performed (on mitochondrial and nuclear datasets separately), after considering pairwise F_{ST} values and significance. The specimens from seeps from Regab area (Regab, and Baboon for mtDNA) were defined as a first group, and the ones from the Congo lobes as a second group.

3. Results

3.1. Diversity

A total of 5 haplotypes (*COI haplotypes*) were recovered from the 176 individuals for which a sequence was recovered. The most common haplotype was found as dominant across all sampled sites and the second most common one was found in all but one site (Baboon, n=4), and is 1 point mutation away from the most common haplotype. The most common haplotype appears central to the haplotype network, the most divergent haplotypes are 3 points mutations

away from the main one: one was specific to the Regab pockmarck. The two other haplotypes, 2 points mutations distant from the major one, were only found in the Lobe A.

Haplotype diversity (*h*) was low, ranging (except for Baboon where the same haplotype was found for the only 4 specimens sampled) from 0.43 in Lobe B to 0.55 in Lobe A, and the nucleotide diversity (π_2) was also low, ranging from 0.0007 in Lobe B to 0.0016 in Regab_SW (Table 2).

No significant departure from neutrality or demographic stability expectation was detected using Fu and Li and Tajima's D statistics, either at the location or at the groups (Regab versus Lobes) levels.

Multilocus genotypes at the eight microsatellite loci analysed for a total of 177 individuals, from the two sites in the West African Equatorial margin, revealed relatively high genetic diversity. The mean number of alleles per locus varied from 5.6 (Lobe B) to 12 (Regab_SW), increasing with sample size (Table 2). Standardized allelic richness across all loci (A_{rich}) ranged from 43.85±0.07 (Lobe F) to 51.95±0.10 (Regab Centre). Unbiased heterozygosity (H_E) varied between 0.78 (Regab_SW, Lobe F) and 0.83 (Lobe B) and the observed heterozygosity (H_O) varied between 0.73 (Lobe F) and 0.85 (Lobe B). A significant departure from the Hardy-Weinberg equilibrium (heterozygote deficiency) was found for the south-western part of Regab pockmark (0.052).

3.2. Differentiation

The mtDNA and microsatellites datasets revealed significant genetic differentiation between locations from the Regab cold seep and the Congo Lobes B and C, while Lobe A and

B showed significant differentiation only for mtDNA. The differentiation between Regab sites was milder with Lobe C (between 0.007 and 0.07 for microsatellites and mtDNA, respectively) than Lobe B (between 0.02 and 0.38 for microsatellites and mtDNA, respectively) (Table 3).

The AMOVA per group showed a significant F_{ST} of 0.12 ($p<0.01$) between Regab and Lobe areas for mitochondrial DNA, while no grouping resulted in any significant AMOVA when based on microsatellites data (Table S2).

4. Discussion

The phylogeny of vesicomyid bivalve species suggested the importance of depth in the diversification process (Decker et al., 2012a; Johnson et al., 2017). The bivalve *Christineconcha regab* was observed at rather high densities at widely separated localities, from about 3000 to 5000 m depth, along the African continental margin. This species is dominant at the most active part of the Regab pockmark (about 3000m depth), and in the more labile habitats formed by the terminal lobe area of the Congo deep-sea fan (about 5000m depth) currently receiving high organic matter inputs (Decker et al., 2017). Yet, despite this relative abundance, results showed a limited genetic diversity (with only 5 mitochondrial haplotypes and limited heterozygosity at microsatellites; Table 2) and connectivity (Table 3) at local scale, among lobes and the distinct part of the giant pockmarck Regab. Nevertheless, hints of differentiation could be observed along the depth range explored here, between Regab and the best sampled fan of the Congo River (Lobe C) as well as the most demographically declining *C. regab* area (Lobe B, Table 1).

In the absence of temporal fluctuation or significant variance in reproductive success, high population densities might reflect large effective population sizes. *C. regab* can settle in cold seeps and the reduced sediments of the Gulf of Guinea where densities as high as 1400 ind.m-² in the Lobes area, and from 500 to 1000 ind.m-² in the Regab pockmark (Decker et al., 2012b; Decker et al., 2017; Olu et al., 2017). These studies revealed that, in the canyon of the Congo deep-sea fan, *C. regab* and *A. southwardae* co-occurred in mixed patches, but in various densities and proportions. The authors showed that, at Lobe B, not currently located in the axis of the Congo channel, a patch of about 91 ind.m-^{2} with both species was found, where *A. southwardae* dominated and *C. regab* is very scarce (about 11.83 ind.m-^{2}, Table 1). In contrast, at Lobes A, F and C located in the axis of the Congo channel, *C. regab* was always the dominant species. At Lobe A, vesicomyids formed dense aggregates of about 566 ind.m-^{2} along the southern slope of the canyon in the "Vesico Bay" area. At Lobe F, vesicomyids were found in low density (about 26 ind.m-^{2}) surrounding microbial mats on the top of a small hill. At Lobe C, vesicomyids were found in a very dense bed (about 1166 ind.m-^{2}), in a heart-shaped patch.

Here, despite the midterm instability expected in the dynamic sedimentary systems formed by the fans of the Congo river, we observe a relationship between the density of local *C.regab* beds (Table 1) and their genetic diversity as revealed by mtDNA and microsatellites (Table 2). In fact, the oldest fan located slightly aside from the main channel (Lobe B), where smaller and sparser vesicomyid beds were encountered, exhibits low levels of diversity at both markers. A similar observation was made for microsatellites for the Lobe F, placed in the middle of the channel and sharing a similarly low density and associated sampling. Nevertheless, no significant results were obtained with bottleneck tests at any of the two markers type used. This may reveal demographic events that are too recent to be detected with the statistical power delivered by the data gathered, or global stability of the system at the metapopulation scale.

277 At the scale of the Lobes area, only hints of mtDNA differentiation between Lobe A and B were
278 detected, suggesting either a stochastic effect due to low sample size for Lobe B, or slight
279 genetic differentiation possibly due to the decreasing demographic trends of the declining and
280 disconnected Lobe B, rather than a real lack of connectivity at local scale. Invertebrates such
281 as vesicomyids may be subject to chaotic genetic patchiness, i.e., small-scale mosaic of genetic
282 differentiation due to local drift and/or the patchy recruitment of larval pool genetically related
283 (Hedgecock, 1994; Broquet et al., 2013). Would such differentiation be real rather than a
284 statistical artefact due to low sample size, it would not be surprising to better detect such pattern
285 with markers exhibiting lower effective population size than nuclear ones, such as those based
286 on mitochondrial DNA. The Lobe B exhibits rather consistent values of F_{st} (Table 3) which
287 distinct level of significance may be due to limited statistical power to detect differentiation
288 with low sample size. In fact, it shows significant differentiation with the two sites having
289 average to high sample sizes (>20), and non-significant F_{st} yet similar indices of differentiation
290 with others. Except for the Lobe B, the overall lack of significant genetic differentiation across
291 Lobes of the presently active areas suggests the existence of a single panmictic population at
292 the scale of the Lobes area, with a genetic pool locally shaped by the constant geophysical
293 rearrangement of the channels and sedimentation paths.

294 Factors such as fecundity, timing of reproduction, type of larval development, mortality and
295 oceanic currents all play a role in effective dispersal (Scheltema, 1986). Panmictic populations
296 are generally expected for species with long-range dispersal capabilities and variability in the
297 larval duration. Yet studies about the reproductive biology of Vesicomyidae bivalves suggest a
298 possibly lecitotrophic development (Parra et al., 2009), usually assumed to characterize poor
299 dispersers (Shilling and Manahan, 1994). Data about the larval dispersal duration of *C. regab*
300 are not available, but high reserves in the eggs may represent an advantage by providing

nourishment during long-distance dispersal across inhospitable habitats. Besides, contrarily to other bivalve species exhibiting a fixed adult stage, vesicomyid clams have an adult mobile lifestyle that enables them to maintain their population in the ever-changing landscape of sulphide-rich sediment outcrops (Sen et al., 2017) and may contribute to dispersal. Both dispersal capacity at larval stage and the ability of adults to move away from environmental conditions when they become less suitable (as in Lobe B now out of the main channel axis), may thus explain the lack of bottleneck and genetic panmixia observed at the scale of the Lobes area despite an environment characterized by periodic changes and instability.

At regional scale, a consistent differentiation was found with both nuclear and mitochondrial markers between the well-sampled site of SW-Regab, and both Lobes B and C, whereas the Lobe A at the entrance of lobes areas exhibits non-significant but also much lower values of F_{st}. The detection of significant levels of differentiation between vesicomyids bed of Regab pockmark and those downstream lobes may be related to their activity, position in the channel, and sampling density. In fact, despite similarly low sampling density at Lobes B and F, only Lobe B stands with significant differentiation values, which suggests a stronger signal of differentiation due to its remnant situation outside the present network of channels and its lower population density. As for the Lobe C where most activity is detected, it is also the one with the highest sampling density, thus increasing the power to detect significant differentiation even with more moderate levels of restriction to gene flow.

Contrastingly, the lack of differentiation of the upper stream Lobe A suggests present or recent connectivity with both lobes and pockmark areas. Currents likely decrease at the lobe entrance (Vangriesheim et al., 2009), which may influence the demographic trends and connectivity, and result in slightly distinct distributions of genetic polymorphism. In fact, at sites A and F, where a clearly defined channel is present, clams were absent from within the channel and almost only

detected in the lobes themselves. At site C, the channel is wider, and currents are slower, which, combined with higher sedimentation rates, allows for the establishment of more sulfide-rich habitats suitable for clam colonization. In fact, patches of black reduced sediment were much more abundant at site C, despite their occupancy by vesicomyids was very low (only 9% compared to 21% at site F and 91% at site A) (Sen et al., 2017). Furthermore, the lobe around Site A is likely the oldest and the lobe around Site C the youngest (Babonneau et al., 2002; Dennielou et al., 2017; Savoye et al., 2009), a situation that may contribute to explaining their contrasted patterns of differentiation with upper pockmark populations.

Here, we show relative genetic homogeneity across a small spatial scale despite apparent chaotic patchiness for some markers, with differentiation at the scale of hundreds of kilometers, among sites also located at different depths. Population genetics studies in the deep-sea are still scarce (Taylor and Roterman, 2017), yet some echoes our findings. Most report a lack of genetic differentiation among populations of species inhabiting hydrothermal-vent or cold seeps, at regional scale (Mussels: Thaler et al., 2017; Cowart et al., 2013; Cowart et al., 2014), and at large scale (Mussels: DeLeo et al., 2022; Annelids: McMullin et al., 2010; Gastropods: Thaler et al., 2011; Crustaceans: Teixeira et al., 2012). Nevertheless, some of those studies also show, as the present one, the emergence of genetic differentiation at large spatial scale, such as the case of the tubeworms of the genus *Escarpia* (Cowart e al., 2013), or hydrothermal vent shrimp (Teixeira et al., 2013). Among the few cases of genetic differentiation, some were detected using SNPs and mitochondrial markers (Crustaceans: Cheng et al., 2020). Moreover, the analysis of SNPs in the Squat Lobster *Shinkaia crosnieri* revealed hundreds of genes under potentially positive selection across depths, possibly suggesting local adaptation to distinct environmental conditions (Xiao et al., 2020). The existence of differences in developmental processes and depth-related and metabolic adaptations to chemosynthetic environments have

been proposed, that provides clues into species-specific adaptations that enable survival and potential speciation within such ecosystems (DeLeo et al., 2022). Similarly, the present study reports indices of genetic differentiation across sites separated by several hundreds kilometers, an effect confounded with that of depth. This study thus calls for future analysis including more sites located at different depth, and providing genome scans, to further understand the role of distance and/or depth on genetic differentiation as well as the relative incidence of drift-migration *versus* selection on those processes.

Conclusion and perspectives

Several authors have argued in favour of a depth-segregation hypothesis of vesicomyid bivalve species (Olu et al., 1996; Kojima and Ohta, 1997; Goffredi et al., 2003), mainly driven by species-specific features of egg buoyancy, larval development and dispersal, but possibly also due to other pressure-related physiological or biochemical adaptations of juveniles and adults (Goffredi et al., 2003). In the Gulf of Guinea, *C. regab* was both found in pockmarks (3000 m depth) and deep lobes area (5000 m depth), adding to previously reported examples of relatively large depth range (Cosel and Olu, 2009). Although our results are generally in agreement with medium (tens to hundreds km) distance dispersal among *C. regab* beds, we also find evidence of some genetic differentiation at various spatial scales. The significant genetic differentiation found between Regab cold seep and the B and C Lobes with the intermediate position of the upper stream Lobe A might reflect isolation by depth mostly detectable in situations where statistical power is higher (high signal and/or large sampling size, respectively). At local scale, the irregular pattern of F_{st} across lobes areas suggests chaotic genetic patchiness, possibly due to sedimentation triggering environmental instability and recurrent local extinctions-

372 recolonization of the local demes. This would cause an instant drift in vesicomyid beds, and
373 may explain the heterogeneous hints of differentiation observed among lobes and among Regab
374 and lobes. Due to the remote and difficult access to the deep sea, low sample size unfortunately
375 characterizes many collections and limits the conclusion of subsequent studies. The increasing
376 access to genome sequencing and high-density genome scans will ultimately allow partly
377 compensating this limitation. Not only will genome scan may partly compensate for low sample
378 size (Pritchard et al., 2000) but also in cases such as the one of *C. regab*, it to better elucidate
379 the respective roles of drift and possible selective processes due to high environmental
380 instability and heterogeneity. Such studies would, however, require further exploration to better
381 understand the habitat distribution of *C. regab*. This species is known to be present mostly in
382 the sites included in this manuscript, despite some ancient record (1974) support its occurrence
383 in the Bay of Biscay (Krylova and Cosel, 2011) where sampling would allow moving further
384 in this direction.

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Author contributions

K.O. and S.A.H. designed the study. K.O., C.D., O.M. and S. A. collected the field data. S.T and S.F. produced the genetic data. S.T, S.A.H. and M.H. analysed the data. S.A.H. contributed with reagents/ materials/ analysis tools. M.H., S.T., K.O. and S.A.H. interpreted the data and wrote the article. All authors critically revised the manuscript.

Data Accessibility

DNA sequences: GenBank accessions: *Christineconcha regab* COI haplotypes: OP550437 through OP550441.

672 Microsatellites and haplotypes genotypes data are available on the repository SEANOE

673 (https://www.seanoe.org/) under the doi: https://doi.org/10.17882/94263.

Tables

Table 1: Geographic regions, GPS coordinates and depth of sample recollection, and main features of congolobes (according to Rabouille et al., 2017), together with mean density of *C. regab* and average size in each of the sampling location (Decker et al., 2017).

Cruise	Year	Area	Site	Latitude	Longitude	Depth	Main features	Density (all vesico with sd)	Proportion *C. regab*	Density (ind.m-2)	Average size (SE)
WACS	2011	Regab	South West	5° 52.813' S	9° 37.942' E	3150	Edge of a stable pockmark	681 (296)	91	647	90.2 (2.2)
			Centre	5° 52.813' S	9° 37.942' E	3150	Center of the stable pockmark	1056 (218)	98.5	1056	84.3 (10.2)
		Baboon		4° 57.000' S	9° 56.460' E	3100	Pockmark cluster				
CONGOLOBE	2012	Congo lobes	Lobe A	6° 27.000' S	6° 2.000' E	4770	Entrance of the channel	566 (261)	92	520.72	73.2 (8.9)
			Lobe B	6° 25.000' S	6°49.000' E	4880	Away from active area	91 (30)	13	11.83	70.5 (4.8)
			Lobe C	6° 41.000' S	5°28.000' E	4950	Main deposition zone, high sedimentation rate	1166 (361)	98	1142.68	60.2 (11.5)
			Lobe F	6° 35.000' S	5°41.000' E	4820	In the channel	26 (15)	61	15.86	64.8 (5.6)

Table 2. Genetic diversity indices of *C. regab* sampled in the West African Equatorial margin, estimated for each sampled site from mitochondrial COI and 8 microsatellites for sample size *n*; parameters obtained for mtDNA are detailed as follows: (*k*) number of polymorphic sites; (*N*h) number of haplotypes; (*h*) haplotype diversity (±SD); (π_1) mean number of pairwise differences; (π_2) nucleotide diversity. Neutrality and population expansion tests: *D*= Tajima's D-test; F_S= Fu's F_S test. For microsatellites data, mean number of alleles across loci (*A*), A_{rich} standardized allelic richness for 7 individuals across 8 loci (±SE), observed (H_O) and expected (H_E) heterozygosities and heterozygote deficiency (F_{IS}) are detailed. Asterisk indicate significant values ($^{***}p<0.001$) after q-value correction.

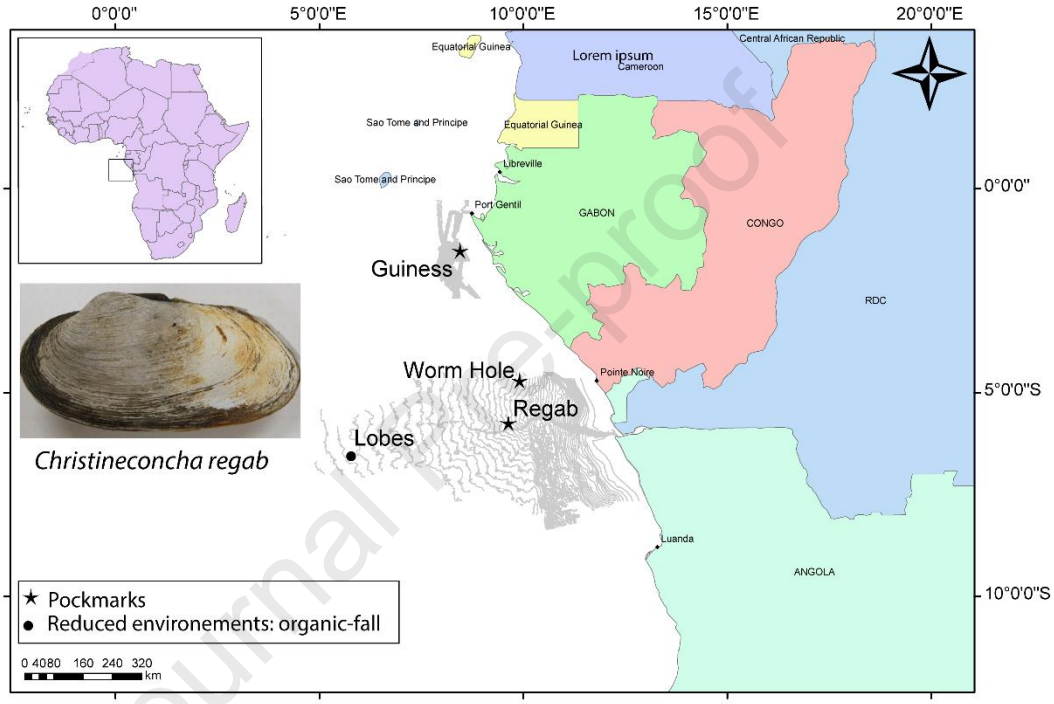
Site	Depth (m)	$_{mt}$DNA								microsats					
		n	*k*	*N*h	*h*	π_1	π_2	*D*	F_S	n	*A*	A_{rich}	H_E	H_O	F_{IS}
Regab Area															
Baboon	3000	4	0	1	0	0	0	-	-	-	-	-	-	-	-
Regab_SW	3150	66	4	3	0.44±0.07	0.91	0.0016	0.18	2.05	60	12	48.67±0.11	0.78	0.74	0.052*
Regab Centre	3150	11	4	3	0.47±0.16	0.87	0.0015	-1.32	0.32	11	8.12	51.95±0.10	0.79	0.76	0.038
Congolobes															
Lobe A	4770	20	3	4	0.55±0.11	0.77	0.0013	-0.24	-0.61	24	10	47.52±0.12	0.80	0.79	0.006
Lobe B	4820	8	1	2	0.43±0.17	0.43	0.0007	0.33	0.54	7	5.6	45±0.07	0.83	0.85	0.000
Lobe C	4950	59	1	2	0.48±0.03	0.48	0.0008	1.61	1.96	66	11.5	47.52±0.10	0.80	0.80	0.000
Lobe F	4880	8	1	2	0.54±0.12	0.54	0.0009	1.17	0.87	9	6.25	43.85±0.07	0.78	0.73	0.070

Table 3. Pairwise F_{ST} estimator of genetic structure θ computed from GENETIX on the basis of haplotypes and allele frequencies (upper for microsatellite loci, lower for mtDNA data). Significant levels are indicated (* p<0.05; **p<0.01).

	Regab SW	Regab Centre	Lobe A	Lobe B	Lobe C	Lobe F
Regab SW		0.006	0.002	0.020*	0.007**	0.010
Regab Centre	0.000		0.005	0.025	0.013*	0.000
Lobe A	0.006	0.000		0.009	0.000	0.002
Lobe B	0.380**	0.336	0.270*		0.016	0.004
Lobe C	0.071**	0.071	0.016	0.167		0.011
Lobe F	0.013	0.014	0.000	0.143	0.000	

Figure 1. Location of the western African sites illustrating a) the position of chemosynthetic ecosystem associated to pockmarks and massive organic falls in the lobes (modified from Decker, 2017) and b) the region of Congo deep-sea fans, with limits of the lobes indicated by dashed lines, and numbers from the oldest (1) to the youngest (5), modified from Rabouille et al. (2017).

a)



b)

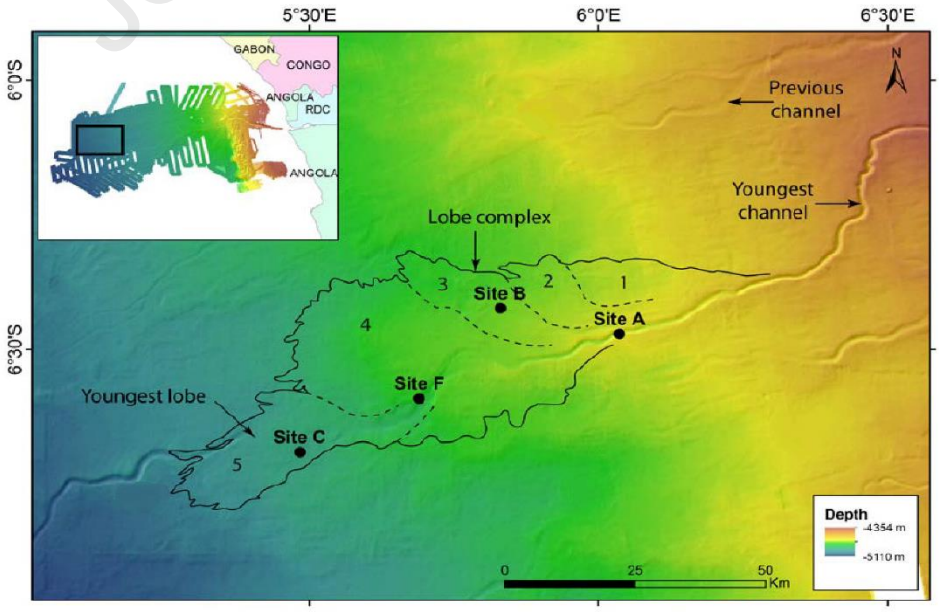
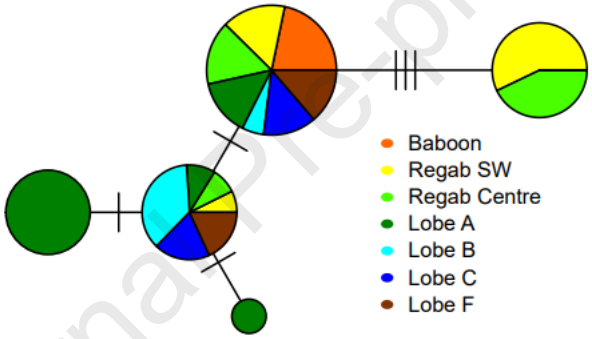


Figure 2. Haplotype networks of the mtDNA haplotypes (580 bp) obtained for *Christineconcha regab* bivalves. Each circle represents a different haplotype, with the size of each circle proportional to the number of individuals displaying that particular haplotype. The colours used represent the locations where the haplotypes were found and within pie charts, the segment size is proportional to the relative frequency of a haplotype in each population where it is present. Mutation steps are represented only when higher than 1.



Declaration of competing interest

Dear Editor,

We would like to submit the attached manuscript entitled **"High connectivity among Vesicomyid bivalves from cold seeps and deep-sea fan of Congo"** to **Deep-Sea Research I.**

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Yours sincerely.

Mohamad Hassan and Sophie Arnaud-Haond