Reconstructing the dynamics of past coral endosymbiotic algae communities using coral ancient DNA (*cora*DNA)

Olivier Rey [1], Delphine Dissard [2], Eve Toulza [1], Thomas Guinebert [3], Mathilde Saccas [3], Jean-François Allienne [1], John Butsher[2], Mourad BenSalah Zoubir [4], Iwankow G. [5], Christelle Tougard [6], Jérémie Vidal-Dupiol [3]

[1] IHPE, Univ Montpellier, CNRS, IFREMER, Univ Perpignan Via Domitia, Perpignan, France

[2] IRD/UMR LOCEAN (IRD-CNRS-MNHN-Sorbonne Université), Centre IRD de Nouméa, 101 Promenade Roger Laroque, Nouméa 98848, New Caledonia.

[3] IHPE, Univ. Montpellier, CNRS, IFREMER, Univ. Perpignan Via Domitia, Montpellier France

[4] Radiology Department, Centre Hospitalier de Perpignan, Perpignan, France

[5] PSL Research University: EPHE - UPVD - CNRS, USR 3278 CRIOBE, 66860, Perpignan, France

[6] ISEM, Université de Montpellier, CNRS, IRD, EPHE, Montpellier, France.

Abstract

Most scleractinian corals are drastically threatened due to global changes but some colonies are intriguingly resistant to heat stress. Coral thermal tolerance partly relies on genomic determinism among the cnidarian compartment but also on the physiology of their associated symbiotic algae (Symbiodiniaceae). In fact, some corals can shift and/or shuffle their associated Symbiodiniaceae communities to temporally cope with heat stress. So far coral adjustments of their endosymbiotic algae were mainly observed at short-term evolutionary time scales and we lack a general vision of coral holobiont evolution at broader timescales. We here combined the use of ancient DNA from a coral core and a metabarcoding approach, to retrace past Symbiodiniaceae communities associated with a living colony of *Porites lobata* from New Caledonia over the last century. We were able to extract ancient DNA along the coral core at 19 time points dating back to the 1870's. Overall, we detected 13 OTUs, nine of which were affiliated to the Symbiodiniaceae *Cladocopium* clade, one to *Azadinium spinosum* (Dinophycae); one to the host *P. lobata*, the two other OTUs remained unidentified. One OTU was largely predominant and was ubiquitous over all samples. The number of OTUs was marginally correlated to the total number of sequences per sample but not to the age of the *cora*DNA sample. We found a generally stable core microbiota associated with *P. lobata*, although drastic change in community composition was observed in coraDNA samples corresponding to an extreme hot winter temperature event. More generally, this study paves the way for further investigations on the evolutionary dynamics of coral holobionts at the colony level over large temporal scales.

Keywords

Coral; Symbiodiniaceae; Ancient DNA; metabarcoding, *ITS2*

Introduction

Scleractinian corals and the associated reef ecosystems are among the most threatened systems worldwide due to global changes. Almost half of all living corals have already been destroyed in the last 150 years [1]. Such massive loss has accelerated over the last three decades under the influence of extreme climatic events, in particular heat waves, the frequency of which is steadily increasing [1]. Despite this alarming situation, coral colonies particularly resistant to heat stress have been identified [2–4]. These observations provide some hope for the maintenance and/ or the restoration of corals and ecosystems they support. They also call for an urging need to unravel the molecular mechanisms by which some coral colonies survive through time despite recurrent events of environmental stresses and help adjust conservation plans. This task is all but trivial in particular because corals are complex holobionts composed of cnidarians associated with microbial communities including – among others – endosymbiotic algae (Symbiodiniaceae), protista and bacteria, each of these partners potentially influencing the thermotolerance of the colonies [5,6].

Variation in coral thermal tolerance across latitude, at least partly relies on genomic variation among the cnidarian compartment of coral colonies [7]. In this respect, it has been suggested that resistant genotypes could emerge through intrinsic rapid genomic changes such as somatic mutations [8], the activation of transposable elements [9] and/ or some modifications in the proportions of genotypes coexisting within the same colony [10,11]. Additionally to genomic modifications, changes in DNA methylation patterns in cnidarians may also induce – at least temporarily – adaptive phenotypic adjustments in coral colonies exposed to recurrent heat stress [12,13]. Besides changes within the cnidarian compartments, coral thermal tolerance also depends on the physiology of their associated symbiotic algae (Symbiodiniaceae), with which they form a phototrophic mutualistic symbiosis [14,15]. Symbiodiniaceae constitute a highly diversified taxonomic group among which the identified species and even strains among species display huge variation in thermal tolerance [16,17]. Recently, a laboratory experiment has shown that experimentally adapted strains of the Symbiodiniaceae *Cladocopium goreaui* to high temperature, provide a better protection to heat stress in *Acropora tenuis* colonies after reimplantation [18]. This suggests that thermal tolerance may be acquired rapidly by natural coral colonies *via* the acquisition of acclimatized or adapted Symbiodiniaceae from the surrounding environment in response to heat stress. In fact, symbiont switching – the acquisition of new (thermally resistant) species/strain from the environment – and symbiont shuffling – the modification of the relative abundance of the inner Symbiodiniaceae strains within host –

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78   constitute two alternative rapid responses for corals to temporarily cope with heat waves [19,20].

79   Finally, while other partners involved within the coral holobiont such as bacteria, fungi, viruses

80   and protists, certainly display higher adaptive capacity than corals in particular due to their short

81   generation time, their role in fostering adaptive response to heat stress at the holobiont scale

82   still remain to be demonstrated [5,13,21].

83   Most of our knowledge on the mechanisms underlying the adaptive response of coral

84   colonies to global changes relies on empirical studies based on controlled experiments

85   conducted either in laboratory or in the field, over extremely short-term evolutionary time

86   scales. These approaches are crucial to dissect the relative importance of specific molecular

87   mechanisms in action to foster coral responses. They have dramatically changed our vision on

88   the short-term adaptive capacity of corals [12,13]. However, and complementary to these

89   approaches, we need a more general vision of coral holobiont evolution at broader timescales

90   to assess their evolutionary dynamics in response to the recent fast evolving environmental

91   changes. In this respect, ancient DNA (aDNA) based approaches are promising [22]. These

92   approaches generally rely on DNA extracted from archaeological or paleontological remains or

93   from museum samples. For instance, museum samples of 8 octocoral species originally

94   collected from successive epochs revealed that mutualism between several coral species and

95   their associated Symbiodiniaceae remained stable overtime with no major changes in the last

96   two centuries despite major anthropogenic global change [23]. In a more general context of

97   biodiversity, aDNA was also applied to reconstruct the community assemblages of reef

98   ecosystems from sediment cores [24,25]. Surprisingly however, no attempts were made to extract

99   aDNA directly from cores excavated from a living massive coral colony that chronicles decades

100   and up to centuries, of its lifetime. Such an approach is particularly promising because it could

101   allow accessing the DNA material from individual holobiontic colonies over time and thus

102   reconstructing their intrinsic eco-evolutionary history.

103   We here provide the first proof of concept of the use of aDNA from a coral core,

104   hereafter called *cora*DNA (referring to the recent *seda*DNA approach developed to study DNA

105   from sedimentary cores), to reconstruct past Symbiodiniaceae communities associated with a

106   living colony of *Porites lobata* over the last century. We discuss the benefit of such approach

107   to unravel the eco-evolutionary dynamics of coral holobiont and the current technical

108   limitations that will need to be bypassed to have access to DNA from the cnidarian compartment

109   and hence reconstruct the full eco-evolutionary trajectories of coral holobionts over time.

110

111   **Material and methods**

112

113   ***Core sampling***

114   *Porites lobata* is a massive reef-building scleractinian coral ubiquitous over the Tropical Pacific

115   Ocean. Colonies can be extremely long-lived (up to several centuries) and their growth strongly

116   depend on the environmental conditions in which they grow, especially seawater temperature

117   [26]. These characteristics make this species particularly suitable in the context of this study since

118   it makes it possible to obtain long cores and the marked seasonal successions of growth allow

119   the collection of core matrix at the year scale from which DNA can be extracted. A coral-matrix

120   core was collected from a 2.5-m high colony of *Porites lobata* at 10-m depth within the

121   Southern Lagoon of New Caledonia (22°17,146 et 166°11,004) the 17th of December 2018

122   (Figure 1.A.). The excavated 8-cm diameter and ~ 80-cm long core was immediately stored in

123   dry ice and transferred under freezing conditions to the Institute de Recherche pour le

124   Developpement (IRD) in Nouméa, prior being sent in dry ice to the IHPE laboratory in

125   Perpignan where it was stored at -80°C until subsequent analyses. Thus, the core has been kept

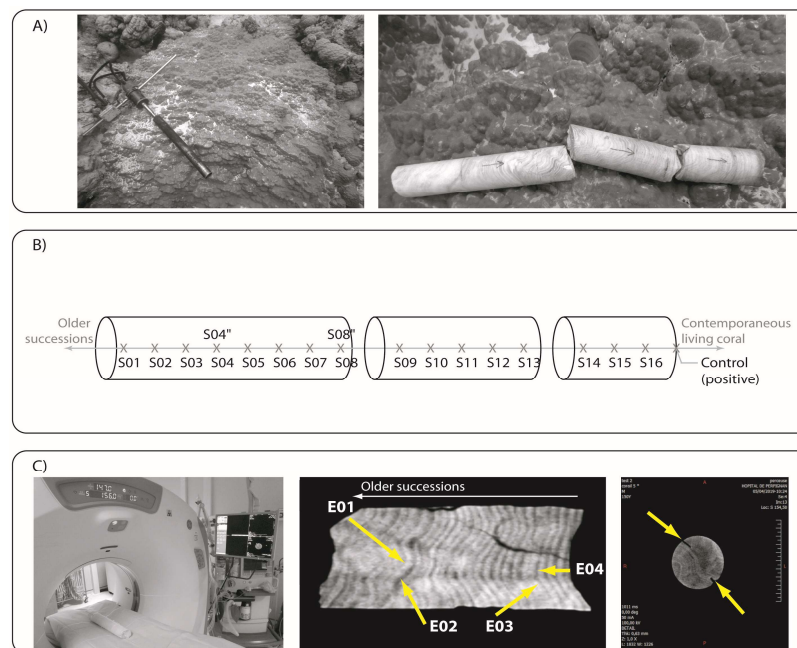126   at -80°C from its initial excavation until the sampling of *cora*DNA.

127

128   ***Figure 1:*** *Details on the coral core of the studied Porites lobata colony. Pictures of its excavation in*
129   *December 2018 from the living colony in New Caledonia are provided in (A). In (B), the coraDNA*
130   *samples that were extracted along the core are represented from the older sample (S01) to the*
131   *contemporaneous living coral (control). In (C) Pictures of the interventional sampling using a CT scan*
132   *of the four coraDNA samples corresponding to successions before (E01) and after (E02) the 1997-1998*
133   *ENSO event; and before (E03) and after (E04) the anormal hot winter in 2010. Yellow arrows in the*
134   *last panel in (C) point toward the hole left by the drilling bit after the sampling of the E04 coraDNA*
135   *sample.*

5

136 *Dating successions and sampling ancient DNA from the coral core*

137 The aging of the coral core was determined based on the counting of density bands as described

138 in Wu *et al.* [27]. Annual skeletal density bands were observed using a CT scan at the imagery

139 service of the public Hospital of Perpignan (France). The observed density bands result from

140 variation in colony growth rate during the winter (slow) and during the summer (rapid) that lead

141 to a more or less dense aragonite skeleton along the year [26]. According to this pattern, one year

142 was reconstructed by summing a clear (summer) and dark (winter) density bands [27]. Based on

143 the reconstructed chronology of the sampled coral colony, a total of 23 samples were collected

144 along the coral core (Figure 1.B and C). Briefly, each sampling location along the core was first

145 rapidly washed using a 10% NaClO solution and ~ 3 mm of the external layer was removed

146 from the core surface using a sterile disposable scalpel to avoid possible DNA contamination

147 with contemporaneous genomic material. Once the surface of the core was decontaminated, an

148 electric driller with an individualized sterile 2-mm diameter drill bit was used to extract core

149 powder along a ~ 1.5 – 2 cm depth drilling hole perpendicular to the surface of the core. The

150 bits containing the powder was immediately transferred and rinsed in a 2 ml Eppendorf

151 (DNA/RNA and DNase/RNase free tube) containing 1 ml of Tissue Lysis buffer ATL from the

152 QIAamp DNA Micro Kit (Qiagen) and stored at 4°C until the DNA extraction process (i.e. at

153 most 24 h later).

154 Sampling along the core was achieved following two strategies. The first strategy

155 consisted in systematically sampling every 5 cm along the core without accurate dating.

156 According to the retraced chronology and to the thickness of the observed density bands, such

157 strategy roughly corresponded to a sample collected every 8 to 10 years back in time over the

158 colony history from December 2018 (collection date) until the 1870's. A total of 17 *cora*DNA

159 samples were extracted following this design including one sample from the top of the coral

160 core (as positive control) which corresponds to the living colony at the time of the core sampling

161 (Sample S01 to S16 and control in Figure 1.B.). This strategy was used to specifically test our

162 ability to detect and obtain processable *cora*DNA samples over large timescales. Moreover,

163 two duplicates were sampled for two random samples (S04 and S08; Figure 1.B.). These

164 duplicates consisted in independent *cora*DNA samples obtained from the same section along

165 the core and using two independent sterile drill bits.

166 The second strategy aimed at studying the dynamics of the algae endosymbiotic

167 communities associated with coral colonies during well documented past extreme climatic

168 events. We targeted two well-known climatic anomalies that were easily observable along the

169 core in 2010 (abnormally hot winter) and in 1997-1998 which corresponded to a severe ENSO

6

170   event [27]. Two samples were collected, one prior and the other after each of these two climatic

171   events (N = 4; Figure 1.C.). For this purpose, and to accurately sample the targeted coral

172   successions, the drilling procedure was achieved under interventional CT 3D scan at the

173   imagery services from the public Hospital of Perpignan. The coordinates of the targeted

174   successions were first retrieved, and the drilling sampling was achieved under interventional

175   scan to ensure that the same succession was sampled within the core during the sampling

176   process (Figure 1.C.). The precautions previously described were taken to avoid contamination

177   (treatment with bleached plus abrasion of the ~3 mm of the external layer of the core and a

178   unique sterile drill bit per sample).

179   Additionally to the overall 23 samples collected along the core, a negative control was

180   prepared at the time of the sampling collection to check for possible contaminations over the

181   whole process from DNA sampling to library preparation. This negative control was obtained

182   following the same protocol as described above except that the drill bit did not touch the core

183   prior to the rinsing step into the Tissue Lysis buffer ATL.

184

185   *DNA extraction*

186   The overall DNA extraction process and DNA amplification steps were performed at the

187   degraded DNA platform (Institut des Sciences de l'Evolution de Montpellier, France;

188   http://club.quomodo.com/plateforme-adn-degrade) offering facilities dedicated to the study of

189   ancient DNA. DNA extractions were performed using the QIAamp DNA Micro Kit (Qiagen)

190   following the "*Purification of genomic DNA from bones*" protocol. Briefly, 25 µl of Qiagen

191   Proteinase K were added to each sample. Samples were then incubated at 56°C with shaking at

192   1200 rpm overnight. The day after, 1 ml of AL buffer was added and samples were incubated

193   at 70°C during 10 minutes to inactivate enzymes. After a centrifugation step at full speed during

194   one minute, the supernatant was transferred to a QIAamp MinElute column. Once the DNA

195   attached on the column membrane (centrifugation step at 8000 rpm for 1 minute), the membrane

196   was successively washed using 600 µl of the AW1 and the AW2 buffer. After these washing

197   steps, the membrane was dried by centrifugation during 3 minutes at full speed. The DNA was

198   eluted using 30 µL of sterile water. The eluted DNA samples were then stored at -20°C until

199   subsequent molecular processes. The quality of DNA extracts wase assessed on a Bioanalyzer

200   High Sensitivity DNA kit (Agilent, USA) and DNA was quantified using a Qubit fluorometric

201   quantification with the ds DNA High Sensitivity assay kit (Thermo Fisher Scientific, USA).

202

203 **_Library preparation and sequencing_**

204 Each DNA sample was used as initial template for amplifying a portion of the _ITS2_ gene using

205 a set of 4 primers derived from the literature [28,29] with Illumina adapters and spacers (Table 1).

206

ITS2-MiSeqNN-F: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNGTGAATTGCAGAACTCCGTG
ITS2-MiSeqNN-R: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNCCTCCGCTTACTTATATGCTT
ITS2-MiSeqNNN-F: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGTGAATTGCAGAACTCCGTG
ITS2-MiSeqNNN-R: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNCCTCCGCTTACTTATATGCTT

207 **_Table 1_**_: Sequence of the 4 primers (including adapters and spacers) used to amplify a ~ 350_

208 _bp fragment of the ITS2 gene_

209

210 Concomitantly, the extraction negative control and a PCR negative control (consisting in

211 distilled water) were used in the same PCR reaction to control for potential contamination

212 during the overall process. PCRs were performed in 50 µL containing 5µL of Buffer 10X PCR

213 Gold (1X), 5 µL of MgCL$_2$ (25 mM), 1 µL of each dNTPs (200 µM), 1.35 µL of primer mix

214 (0.25 µM), 0.25 µL of Taq Polymerase (1.25 units), 4 µL of DNA template and 35.4 µL of

215 distilled sterile water. Amplification was performed using the following PCR conditions: an

216 initial denaturation step at 95°C during 7 minutes followed by 35 cycles each of which

217 constituted of a denaturation step at 95°C during 15 sec, an annealing step at 57°C during 30

218 sec and an elongation step at 72°C during 30 sec. After these 35 cycles, a final elongation step

219 at 72°C during 7 minutes was applied. The amplification was checked by migrating 5 µL of

220 each PCR reaction on a 2% agarose gel stained with ethidium bromide and visualized under

221 UVs. Expected size of the amplicon is around 350 bp.

222 Indexed libraries were generated using the standard Illumina two-step PCR protocol using Q5

223 high fidelity DNA polymerase (New England Biolabs). Paired-end sequencing with a 2x250 bp

224 read length was performed at the Bio-Environment platform (University of Perpignan Via

225 Domitia Perpignan, France) on a MiSeq system (Illumina) using v2 chemistry according to the

226 manufacturer's protocol. Sequencing data are available for download on SRA under the

227 bioproject number ###

228

229 **_Data processing_**

230 The sequence datasets were uploaded to the Galaxy web platform [30] and processed using the

231 Finding Rapidly OTUs with Galaxy Solution (FROGS) pipeline at the GenoToul platform

232 (Toulouse, France) [31]. The first pre-processing step of this pipeline consisted in demultiplexing,

8

233   dereplicating and cleaning all reads. Given the theoretical expected amplicon size (i.e., ~ 350

234   bp) and after a quick overview of the overall amplicon lengths, we kept all sequences which

235   sizes ranged from 150 to 490 pb. These filtered sequences were then clustered using the

236   SWARM algorithm using an aggregation distance set at 1. This iterative clustering approach

237   uses amplicons' homology, structure and abundance hence limiting potential biases generated

238   by other approaches such as *de novo* clustering methods (i.e., input order dependencies and

239   arbitrary clustering) (Mahe et al., 2014). Clusters were then cleaned to remove potential

240   chimeras, singletons and under-represented clusters (i.e. <50 sequences) using VSEARCH

241   (Rognes et al., 2016).

242   Each OTU was identified based on a nucleotide megablast using the online standard

243   database (nt/nr) available from NCBI. To more precisely identify OTUs that were affiliated to

244   the Symbiodiniaceae genus *Cladocopium* (clade C) based on the results from Blast, we next

245   computed pairwise genetic distance between each OTU seed sequence and a subset of

246   sequences from different *Cladocopium* strains available from LaJeunesse *et al.* [32]. Pairwise

247   genetic distances were computed using the 'K80' evolutionary model as implemented in the

248   ape package V5.0 in R [33].

249

250   **Results**

251

252   ***Dating back coral successions along the core***

253   The obtained coral-skeleton core followed the colony's growth axis on more than 2/3 of its

254   length (see Figure 1.C.; middle panel for illustration). We were hence capable to accurately date

255   back the growth successions over the last seven decades, i.e., back to 1950. Density bands along

256   the oldest part of the coral core partly deviated from the central vertical axis preventing our

257   capability to date back accurately the oldest successions. Annual growth bands were

258   approximately 5-6 mm thick and generally homogenous along the core. According to this

259   measure, we estimated that the oldest *cora*DNA sample collected from the core (S01; Figure

260   1.B.) roughly correspond to coral holobiont that lived in the 1870's.

261

262   ***Amplicon sequencing from coraDNA samples, sequence affiliation and identification of***

263   ***endosymbiotic algae communities***

264   Because of low DNA concentrations, DNA quality and quantity could not be assessed from all

265   samples except the positive control. PCR amplicons of expected size for ITS2 Symbiodiniaceae

266   marker were however obtained from all samples. After sequencing and filtration steps,

9

267 sequences were validated from 19 out of the 23 initial *cora*DNA samples including the positive

268 control (Table 2). The final number of filtered sequences obtained from these 19 *cora*DNA

269 samples ranged from 1476 (sample S12) to 76403 (sample S13) (Table 2). Four *cora*DNA

270 samples displayed negligible numbers of sequences (from 9 to 33) and were then excluded for

271 further analyses. Similarly, extraction and PCR negative controls exhibited negligible numbers

272 of sequences (8 and 17, respectively) with an expected size of 300 bp (190 – 450 bp; Table 1),

273 which indicates negligible cross-contaminations during all the process from sampling,

274 extraction to sequencing.

275

Sample	% kept	Paired-end assembled (%)	With 5' primer (%)	With 3' primer (%)	With expected length (%)	Without Ns (%)	Nseq OTU > 50 seq
Samples kept in the analyses							
E01	97.29	77.44	77.38	76.15	75.34	75.34	73241
E02	97.12	57.91	57.87	56.68	56.24	56.24	54571
E03	96.37	73.18	73.13	71.18	70.52	70.52	68484
E04	91.60	48.53	48.52	47.35	44.46	44.46	43454
S01	87.58	75.61	75.58	74.20	66.21	66.21	64448
S04	75.10	59.92	59.90	59.09	45.00	45.00	44203
S04"	85.18	15.65	15.62	15.30	13.33	13.33	12993
S05	4.44	89.98	89.96	89.83	3.99	3.99	3928
S06	67.49	65.52	65.50	63.76	44.22	44.22	42583
S08	96.01	77.93	77.90	75.11	74.82	74.82	71366
S08"	97.56	46.68	46.63	45.66	45.54	45.54	44501
S09	63.04	70.01	69.98	68.80	44.13	44.13	43147
S10	8.32	48.20	48.19	48.09	4.012	4.012	3884
S11	85.80	54.13	54.11	53.04	46.44	46.44	45236
S12	6.98	21.95	21.94	21.90	1.53	1.53	1476
S13	96.83	80.93	80.90	78.79	78.36	78.36	76403
S14	93.24	74.74	74.69	73.05	69.69	69.69	68031
S15	56.67	88.20	88.18	86.22	49.98	49.98	48388
Control	28.60	8.73	8.73	8.66	2.50	2.50	2454
Samples removed from the analyses							
S02	0.02	152.242	152.221	152.075	33	33	0
S03	0.02	58.41	58.406	58.344	9	9	0
S07	0.02	76.544	76.531	76.462	15	15	0
S16	0.02	52.119	52.104	52.046	11	11	0
Negative controls							
Extraction	0.02	45.646	45.644	45.597	8	8	0
PCR	0.15	11.01	11.007	10.998	17	17	0

276

277 **Table 2**: *Details on the obtained and conserved sequences from each coraDNA sample.*

278   The sequences clustered into 13 OTUs. Nine of these OTUS were affiliated to
279   Symbiodiniaceae and all of which were assigned to the *Cladocopium* clade. Based on the
280   genetic distances computed between each of these 9 clusters and *ITS2* sequences from
281   LaJeunesse *et al.* [32], all OTUs were more specifically affiliated to the *ITS2*-C15 type (computed
282   distances ranged from 0 to 0.044; Table S1). Among the 4 other non-specific OTUs, one was
283   affiliated to *Azadinium spinosum* (Dinophycae; OTU_7; Query cover = 100%, E-Value = 6e[-156];
284   Per. Ident. = 98.1%); one to *P. lobata* (the coral host species; OTU_11; Query cover =
285   100%, E-Value = 8e[-175]; Per. Ident. = 100%), and the two others were not successfully affiliated
286   with any identified sequence available from the NCBI nr database (OTU_12 and OTU_13).
287   Sequences of *Azadinium spinosum* (OTU_7) were amplified only in the S08 and its duplicate
288   S08" *cora*DNA samples. Amplified sequences of *P. lobata* (OTU_11) were found in the S01,
289   S08, E02 and E03 *cora*DNA samples although at low abundance (Figure 2; Table S2). Finally,
290   OTUs that were not affiliated with any organism documented in the NCBI database (OTU_12
291   and OTU_13) were present in samples S08 and/or S08" again with low read numbers (Figure
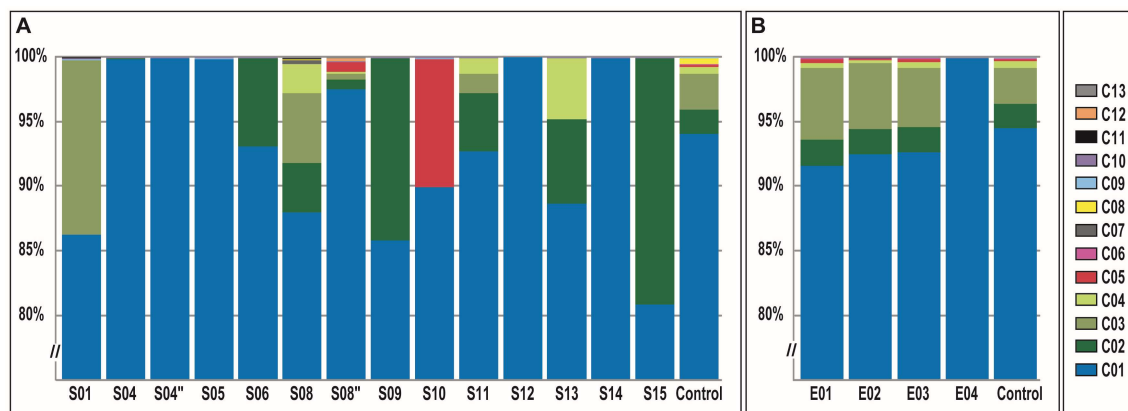292   2).

293

294

295   **Figure 2:** Proportion of sequences attributed to each of the 13 clusters among the 19 positive *cora*DNA
296   samples A. obtained from the systematic sampling (i.e. every 5 cm along the coral core) and B. collected
297   under CT SCAN corresponding to successions before (E01) and after (E02) the 1997-1998 ENSO event;
298   and before (E03) and after (E04) the anormal hot winter in 2010.

299

Distribution of Symbiodinium OTUs over the coraDNA samples

301   The total number of OTUs obtained from each *cora*DNA sample varied from 1 to 10. Among
302   the 9 Symbiodiniaceae OTUs identified, 1 OTU (OTU_1, Figure 2) was clearly predominant
303   representing 92% of the generated sequences overall samples (from 80.88 % in S15 to 100 %
304   in S12). Only OTU_1 is ubiquitous among all *cora*DNA samples. OTU_9 and OTU_10 could

11

305   also be reasonably considered as ubiquitous since they have been detected in all except the S12

306   and the positive control *cora*DNA samples. However, these two samples were the one harboring

307   the lowest sequence coverage which may explain this absence (Table 1).

308   The number of OTUs per sample is marginally correlated to the total number of

309   sequences obtained per sample (Spearman correlation, rho = 0.53; p-value = 0.04) but is not

310   correlated to the age of the *cora*DNA sample (using the position order at which the *cora*DNA

311   was sampled along the core as a proxy; rho = 0.148; p-value = 0.60).

312

313   **Discussion**

314

315   ***Unlocking the access to past coral communities***

316   According to our results, ancient DNA from a coral core (*cora*DNA) excavated from living

317   *Porites lobata* colonies can be obtained and used for metabarcoding approaches to reconstruct

318   the Symbiodiniaceae endosymbiotic communities that have been successively associated to the

319   colonies over a century. This paves the way for unprecedented time-series studies to document

320   the eco-evolutionary dynamics ofthe coral/Symbiodiniaceae mutualistic interaction to most

321   massive coral species.

322   Nine of the 13 OTUs detected in this study belong to the Symbiodiniaceae and all were

323   affiliated to the *ITS2*-C15 type. Since pairwise genetic distances computed between each of

324   these OTUs were relatively low, it is likely that the observed diversity among these strains result

325   from intragenomic variation at the *ITS2* marker as previously highlighted [34,35]. Importantly

326   however, the possible resulting overestimation of the diversity in the Symbiodiniaceae

327   community documented here cannot be related to possible post-mortem DNA modifications

328   commonly observed in ancient DNA samples [36]. Indeed, all but one OTU were detected in the

329   living part of the coral core (positive control) which means that the nature and state of the

330   *cora*DNA samples does not seem to artificially inflate diversity of the Symbiodiniaceae

331   community.

332   Moreover, among the 13 identified OTUs, 1 was attributed to *P. lobata*. This *P. lobata*

333   OTU was detected, although at low abundance, in 4 *cora*DNA samples that do not have obvious

334   commonalities (e.g. spatial location along the core) but a high number of sequences. The

335   detection of the coral host species using the present *ITS2* metabarcoding confirms that host

336   DNA may also be extracted and amplified to some extent. This result makes it possible to

337   consider the exploitation of *cora*DNA samples to trace the eco-evolutionary dynamics of the

338   genome and/or epigenome of the host coral species *P. lobata* at the colony scale.

12

339   Among the OTUs not affiliated to Symbiodiniaceae or to the cnidarian, we unexpectedly

340   detected *A. spinosum* among two *cora*DNA duplicates collected from the same location along

341   the core. These duplicates correspond to coral holobionts that lived in the 1940's – 1950's.

342   *Azadinium spinosum* is a photosynthetic dinoflagellate that was first described in the late 2000's

343   and since then, has been reported on the coasts of Northern Europe [37], South and Central

344   America [38,39] and very recently in Asian pacific [40]. While we found no direct evidence of its

345   occurrence in New Caledonia or the Southern Pacific exists in the literature, *Azadinium spp.*

346   was recently observed in French Polynesia under a scanning electron microscope (Mirielle

347   Chanin; pers. comm). Importantly *A. spinosum* is one of the primary producer of azaspiracid

348   toxins causing important health issues to several animals, mainly vertebrates including humans,

349   through toxin bioaccumulation [41]. In this context, azaspiracid-2 (AZA2) toxins were recently

350   detected in New Caledonia based on a SPATT (Solid Phase Adsorption Toxin Tracking)

351   approach, hence indirectly indicating that *Azadinium spp.* can be present locally at least

352   temporary [42]. So far, no interactions were described between *P. lobata* or any other coral species

353   and *A. spinosum*. We here hypothesize that *A. spinosum* could have been accidentally captured

354   by coral hosts during feeding. Accordingly, the detection of *A. spinosum* at only one time period

355   (although detected in two *cora*DNA duplicate samples from this time period) could coincide

356   with a *A. spinosum* bloom episode as previously described at some localities [38]. Thus,

357   additionally to DNA from Symbiodiniaceae and their coral hosts, our results suggest that we

358   can also detect the punctual presence back in time of other organisms (most likely present in

359   high abundance) in the environment and that may have been captured by corals. However,

360   (short) specific molecular markers tailored to the targeted organisms would be necessary, as the

361   marker used in this study was not initially designed for such purposes.

362   From a technical point of view, it is important to note that the number of OTUs detected

363   in the *cora*DNA samples was associated to sequencing output but not to the age of the *cora*DNA

364   samples (estimated from the position of the *cora*DNA samples along the core). This suggests

365   that a low number of sequences but probably not the age of the *cora*DNA could lead to an under

366   represented vision of the studied past Symbiodiniaceae communities (see also rarefaction

367   curves in Supplementary Figure SF1). This is clearly illustrated by the S12 *cora*DNA fairly

368   recent sample, for which we obtained the smallest number of sequences and that harbors only

369   one OTU (OTU_1; Figure 2) which is also predominant in all *cora*DNA samples (representing

370   80.9 % to 100 % percent of the overall filtered sequences). Conversely, we detected up to 5

371   different clusters in the oldest *cora*DNA sample (S01) including 4 OTUs affiliated to

372   *Cladocopium* and one affiliated to *P. lobata*. We thus advise to sequence libraries from

373   *cora*DNA samples with a high coverage to avoid possible false negatives or to use a rarefaction

374   approach to characterize the optimal sequencing depth.

375

376   ***Dynamics of Symbiodiniaceae at large temporal scale***

377   In addition to breaking down a technical barrier, this study also traces back the dynamics of the

378   Symbiodiniaceae community associated with *P. lobata* over time at the colony scale. More

379   particularly, the Symbiodiniaceae community associated with this *P. lobata* colony is composed

380   of 1 OTU (OTU1) assigned to the *ITS2*-C15-type. This occurrence is largely predominant since

381   the earliest part of the coral core; that was estimated to date back to the 1870's approximately.

382   Two other Symbiodiniaceae OTUs were found to be (nearly) ubiquitous although at very low

383   proportion even in the contemporaneous living coral (OTU9 and OTU10). These three OTUs

384   are thus likely to be in tight association with the coral host and probably part of the core

385   Symbiodiniaceae community of the *P. lobata* colony. This is in line with previous studies that

386   have documented a similar pattern in the symbiotic community associated with natural colonies

387   of *P. lobata* where the Symbiodiniaceae C15 strain was highly predominant [43]. No other clear

388   pattern was observed in terms of community changes over time except for the most abundant

389   non-ubiquitous OTUs such as OTU2, OTU4 and OTU5 which are generally absent in the oldest

390   *cora*DNA samples and appear sporadically only from the S06 – S08 samples (i.e. since the

391   1920's) and upward along the core. Two hypotheses could explain this temporal fluctuation of

392   these OTUs. First, the abundance of these OTUs in the environment display important temporal

393   fluctuations and the studied *P. lobata* colony adjusted its associated Symbiodiniaceae

394   community according to the most prevalent OTU present in the environment. Alternatively, the

395   abundance of the different Symbiodiniaceae strains in the environment is relatively stable in

396   time and the *P. lobata* colony adjusted its Symbiodiniaceae community according to its

397   interaction' preference, physiological state and/or to the prevailing environment [44]. Importantly

398   however, and considering that each OTU is a singular genetic entity, we lack information

399   regarding the functional and physiological consequence of the association between these

400   different *Cladocopium* strains and *P. lobata* to support one of these hypotheses. The use of

401   more resolutive genetic markers could make it possible to better distinguish between different

402   strains from the C15 clade potentially associated with *P. lobata* colonies(ref). Importantly

403   however, and because ancient DNA is more prone to degradation, we could be constrained by

404   the amplicon size. We would thus be more in favor of combining multiple small barcodes rather

405   than using a larger marker.

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407   *Dynamics of Symbiodiniaceae associated with extreme climatic events*

408   No major changes in the Symbiodiniaceae community were observed after the targeted

409   severe ENSO event that occurred in 1997-1998 and which led to a decrease in growth visible

410   on the core, compared to that observed in the *cora*DNA sampled before this event. Two

411   hypotheses might explain this result. First, the targeted ENSO event did not stress the colony

412   enough (e.g. no thermal bleaching occurred) to temporally modify the associated

413   Symbiodiniaceae community. In this regard, and although not comparable to a bleaching event,

414   stability of the Symbiodiniaceae community associated with *P. lobata* during extreme storm

415   events was previously reported on Kiritimati Island in the central equatorial Pacific Ocean [34].

416   More generally, the association between *P. lobata* and their symbionts are generally

417   acknowledged to be stable over time even when facing environmental stress [34,43]. Alternatively,

418   and despite all precautions, we might have missed the succession corresponding to the past

419   coral colony recovering from the ENSO event during the *cora*DNA sampling. Clearly, more

420   coraDNA replicates could have allowed teasing apart these two hypotheses. However, because

421   of the difficulty of sampling only a single succession of coral growth band, and the limited

422   amount of material that can be obtained, replicating coraDNA samples is particularly tedious.

423   One solution could be to use a drill to excavate larger-diameter cores from natural colonies.

424   The most important pattern observed in this study however, concerns the apparent loss of the

425   Symbiodiniaceae community diversity observed post 2010 abnormal warm winter period [27]. In

426   fact, the E04 *cora*DNA sample harbor a very different symbiotic community compared to the

427   3 others recent *cora*DNA samples and to the control. Only 3 Symbiodiniaceae OTUs were

428   detected in this *cora*DNA sample while 8 to 10 were found in the other 3 recent samples and in

429   the contemporaneous control sample. Such low diversity found in E04 cannot be explained by

430   a lower sequencing coverage (Supplementary Figure SF1). One hypothesis could be that this

431   pattern reflects a temporary and partial loss of Symbiodiniaceae diversity by the coral colony

432   during this abnormally warm and stable period over the year. This is in line with a pattern of

433   decreased in symbiotic algae diversity observed in less variable environments in several coral

434   species [45]. Moreover, the remaining 3 OTUs detected in E04 do not correspond to the most

435   abundant ones, which suggest that this loss in *Symbiodinium* OTUs is likely to be non-random.

436   These 3 OTUs are those that are ubiquitous to all *cora*DNA samples. This result hence supports

437   the hypothesis that these 3 OTUs are part of the core Symbiodiniaceae community of the *P.*

438   *lobata* colony irrespective to their (sometimes low) relative abundance among the colony [15].

439

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440 **Conclusion and perspectives**

441

442 Together our results highlight the fact that ancient DNA can be extracted from cores excavated

443 from stony corals, the so-called *cora*DNA, dating back to at least one century. Moreover, among

444 the DNA material extracted from the core, we detected DNA from the coral host species, the

445 dinoflagellate symbionts and a free-living environmental dinoflagellate. This result paves the

446 way for studying the temporal evolutionary dynamics of coral holobionts at the colony scale.

447 Combined with some geochemical analyses on the same samples collected along the coral core,

448 this approach could provide new insights on the mechanisms underlying the adaptive responses

449 of corals to several past stress events including temperature changes, pH and/or chemicals such

450 as heavy metals.

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453 **Supplementary material**

454 Supplementary table S1: Pairwise genetic distances computed between each pair of the

455 sequences from the OTUs obtained in this study or from previously identified *Symbiodinium*

456 strains available from LaJeunesse *et al*. (2003).

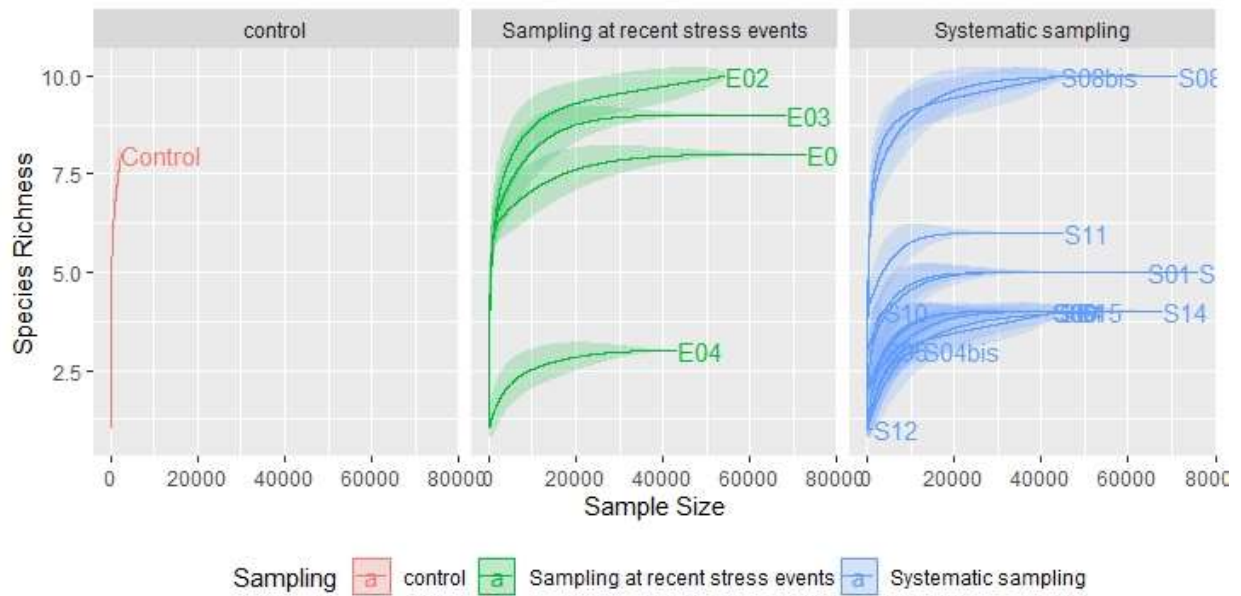|	OTU__1	OTU__2	OTU__3	OTU__4	OTU__5	OTU__6	OTU__8	OTU__9	OTU__10
OTU__1	0	0,014	0	0,007	0,007	0	0,044	0,022	0,022
OTU__2	0,014	0	0,014	0,022	0,022	0,014	0,059	0,036	0,036
OTU__3	0	0,014	0	0,007	0,007	0	0,044	0,022	0,022
OTU__4	0,007	0,022	0,007	0	0,014	0,007	0,051	0,029	0,029
OTU__5	0,007	0,022	0,007	0,014	0	0,007	0,051	0,029	0,029
OTU__6	0	0,014	0	0,007	0,007	0	0,044	0,022	0,022
OTU__8	0,044	0,059	0,044	0,051	0,051	0,044	0	0,067	0,067
OTU__9	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0	0,029
OTU__10	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,029	0
AY239363.1_Symb_C1b	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY239364.1_Symb_C1c	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY239365.1_Symb_C3h	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AY239366.1_Symb_C3j	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AY239367.1_Symb_C8	0,037	0,052	0,037	0,044	0,044	0,037	0,083	0,059	0,059
AY239368.1_Symb_C8a	0,044	0,059	0,044	0,052	0,052	0,044	0,091	0,067	0,067
AY239369.1_Symb_C15	0	0,014	0	0,007	0,007	0	0,044	0,022	0,022
AY239370.1_Symb_C17	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AY239372.1_Symb_C21	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AY239378.1_Symb_C26	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AY258488.1_Symb_C1d	0,036	0,051	0,036	0,044	0,044	0,036	0,082	0,059	0,059
AY258496.1_Symb_C31	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY258501.1_Symb_C35a	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AY765398.1_Symb_C1m	0,037	0,052	0,037	0,044	0,044	0,037	0,083	0,059	0,059
AY765400.2_Symb_C33	0,044	0,059	0,044	0,052	0,052	0,044	0,091	0,067	0,067
AY765401.1_Symb_C35	0,036	0,051	0,036	0,044	0,044	0,036	0,082	0,059	0,059
AY765402.1_Symb_C42	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY765413.1_Symb_C78	0,036	0,051	0,036	0,044	0,044	0,036	0,082	0,059	0,059
AY765414.2_Symb_C79	0,044	0,059	0,044	0,052	0,052	0,044	0,091	0,067	0,067
AF333518.1_Symb_C4	0,014	0,029	0,014	0,022	0,022	0,014	0,059	0,036	0,036
AY589730.1_Symb_C1g	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY589734.1_Symb_C3f	0,029	0,044	0,029	0,036	0,036	0,029	0,075	0,051	0,051
AY589746.1_Symb_C31a	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY589752.1_Symb_C47	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY589771.1_Symb_C66	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY258473.1_Symb_C1h	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AY258475.1_Symb_C8b	0,037	0,052	0,037	0,044	0,044	0,037	0,083	0,059	0,059
AY258481.1_Symb_C37	0,052	0,067	0,052	0,06	0,06	0,052	0,099	0,075	0,075
AY258485.1_Symb_C40	0,036	0,051	0,036	0,044	0,044	0,036	0,082	0,059	0,059
AY258487.1_Symb_C42	0,036	0,051	0,036	0,044	0,044	0,036	0,082	0,059	0,059
AF499789.1_Symb_C3	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AF499794.1_Symb_C4	0,029	0,044	0,029	0,037	0,037	0,029	0,075	0,051	0,051
AF499797.1_Symb_C7	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
AF499801.1_Symb_C12	0,022	0,036	0,022	0,029	0,029	0,022	0,067	0,044	0,044
JQ003816.1_Symb_D	0,827	0,82	0,827	0,856	0,827	0,827	0,856	0,82	0,861

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459 Supplementary table S2: Raw data of the sequence abundance of each OTU identified in this

460 study over all *cora*DNA samples.

OTU_id	Nseq total	S01	S04	S04"	S05	S06	S08	S08"	S09	S10	S11	S12	S13	S14	S15	E01
OTU_1	748108	55576	44178	12986	3924	39658	62753	43406	37033	3493	41942	1476	67729	68005	39137	6710
OTU_2	32296	0	1	0	0	2912	2786	340	6099	0	2030	0	4994	3	9241	1460
OTU_3	23547	8773	0	0	0	0	3858	172	0	0	694	0	0	0	0	4078
OTU_4	6503	0	0	0	0	0	1574	84	0	0	553	0	3653	0	0	237
OTU_5	1193	0	0	0	0	0	0	333	0	386	0	0	0	0	0	225
OTU_6	264	0	0	0	0	0	0	0	0	0	0	0	0	0	0	123
OTU_7	230	0	0	0	0	0	211	19	0	0	0	0	0	0	0	0
OTU_8	141	0	0	0	0	0	113	0	0	0	0	0	0	0	0	0
OTU_9	139	8	11	5	1	6	10	1	9	2	9	0	15	15	6	7
OTU_10	114	10	13	2	3	7	8	8	6	3	8	0	12	8	4	4
OTU_11	92	81	0	0	0	0	4	0	0	0	0	0	0	0	0	0
OTU_12	94	0	0	0	0	0	0	94	0	0	0	0	0	0	0	0
OTU_13	93	0	0	0	0	0	49	44	0	0	0	0	0	0	0	0

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463   SF1: Rarefaction curves obtained for each of the 19 samples.

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Declarations

Availability of data and materials

Raw sequences from the metabarcoding approach are available on GenBank under the SRA project id. PRJNA1086717.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

O.R and J.V.D equally contributed to the conception and design of the work. The coral core was sampled and sent to the IHPE laboratory by D.D and J.B with the help of G.I for administrative aspects. D.D. and T.G. dated the coral core successions. T.G, M.S, O.R, M.B.Z and J.V.D acquired the ancient DNA extracts with the help of C.T at the degraded DNA platform (Montpellier). O.R, J.F, M.S and J.V.D processed the DNA extracts. O.R., T.G and E.T. analyzed the metabarcoding datasets. O.R., T.G. and J.V.D interpreted the results. O.R have drafted the manuscript and J.V.D, D.D and E.T. substantively revised it. All authors approved the submitted version.

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