



Etude des mécanismes qui génèrent et maintiennent la biodiversité en milieu marin: le cas des invertébrés à fort pouvoir dispersif

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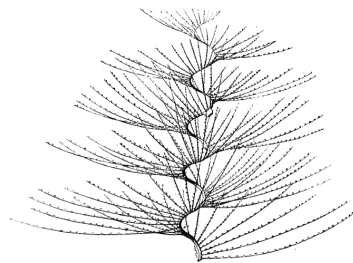
Etude des mécanismes qui génèrent et maintiennent la biodiversité en milieu marin: le cas des invertébrés à fort pouvoir dispersif

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UMR 7266 CNRS - Université de La Rochelle



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Mémoire présenté en vue de l'obtention de l'Habilitation à Diriger des Recherches (HDR)

Jury:

- Pr. Didier Bouchon (examinateur)
- Pr. Emmanuel Douzery (rapporteur)
- Dr. Olivier Geffard (rapporteur)
- Dr. Didier Jollivet (rapporteur)
- Dr. Pierre-Guy Sauriau (examinateur)

Remerciements

Je tiens à remercier chaleureusement les personnes qui m'ont fait découvrir les mondes de la recherche et de l'enseignement. J'ai une pensée particulière pour Isaure de Buron, qui m'a pris sous son aile à mon arrivée aux Etats Unis. Je remercie mes collègues et amis Phil Dustan, Allan Strand, Gorka Sancho et toute l'équipe du Grice Marine Lab ainsi que du département de Biologie du College of Charleston. Je remercie mon directeur de thèse Scott France pour son grand sens de la pédagogie, et pour m'avoir donné l'opportunité de développer mon propre sujet de recherche, ainsi que le département de Biologie de l'Université de Louisiane à Lafayette. Je remercie chaleureusement mes camarades de paillasse, Jana Thoma, Mercer Brugler et Esprit Heestand.

J'ai initié au début de mes études doctorales, en 2006, des collaborations avec le Museum national d'Histoire naturelle (MNHN) de Paris, collaborations qui perdurent à ce jour. Je souhaite exprimer toute ma gratitude à Sarah Samadi pour sa bienveillance, son soutien, et tous les conseils qu'elle m'a apporté durant les 12 dernières années. Sarah m'a apporté les outils (tant matériels qu'intellectuels) pour développer ma recherche sur la biogéographie des invertébrés en milieu profond. Une des pierres angulaires de ce travail est la participation aux campagnes Tropical Deep Sea Benthos, organisées par le MNHN et l'IRD. Je remercie les chefs de mission de ces campagnes des nombreuses invitations dont j'ai bénéficié.

Je remercie les membres du laboratoire LIENSs, pour leur soutien durant mon post-doctorat et les multiples préparations au concours de CR au CNRS. Je remercie tout particulièrement Pascale Garcia et Vanessa Becquet pour leur soutien et leurs conseils. Merci aux membres de l'équipe AMARE et à la direction du laboratoire LIENSs. Merci également aux étudiants qui ont participé à la production des données présentées ici.

Mes amis et ma famille m'ont apporté un soutien indéfectible pendant les longues années qui ont précédé l'obtention de mon poste de chercheur. Merci à Amélia et Loup, à mes parents et beaux-parents. Merci à Fanny, Léna et Maeva.

Enfin, je remercie chaleureusement les membres du Jury d'avoir pris de leur temps pour évaluer cette synthèse.

Table des matières

1. Parcours universitaire	4
2. Thématiques de recherche	6
3. Travaux antérieurs	7
a) Écologie des populations de parasites	7
b) Écologie des populations et communautés en milieu récifal	7
c) Biogéographie des coraux de grandes profondeurs	9
d) De la macro-évolution des coraux à la micro-évolution des mollusques	12
4. Programme de recherche	14
a) Introduction	14
b) Projets en cours et perspectives	19
5. Encadrement et enseignement	32
a) Encadrement de thèse	32
b) Enseignement	35
6. Collaborations	36
7. Expertise	36
8. Synthèse	37
Références	37
Annexes	41

1. Parcours universitaire

Après un D.E.U.G. de Biologie obtenu à l'Université Louis Pasteur de Strasbourg, j'ai poursuivi mon cursus universitaire aux États-Unis. J'ai obtenu un Bachelor of Science (B.S) en biologie marine après un an au College of Charleston (Caroline du Sud). Durant cette période, Isaure de Buron et Scott France m'ont initié à la recherche, grâce à un projet indépendant visant à détecter les premiers stades d'infection parasitaire chez le cloporte, à l'aide d'outils moléculaires.

A la suite de ce séjour, j'ai cherché un stage de recherche en France, afin de commencer à construire un réseau professionnel, et découvrir le monde de la recherche française. J'ai donc passé quelques mois à l'EPHE de Perpignan pour travailler sur le recrutement des coraux scléactiniaires, sous la direction de Mehdi Adjeroud.

Au terme de ce stage, je suis retourné au College of Charleston pour travailler sur mon Masters of Science. J'ai intégré la station marine de Grice ("Grice Marine Lab") pour travailler avec Phil Dustan sur la structure spatio-temporelle des coraux aux Bahamas. Au démarrage du programme, le sujet de thèse n'était pas défini. J'ai donc construit un projet avec Phil, et déposé des demandes de financement pour analyser sur les causes de l'effondrement des communautés de coraux scléactiniaires aux Bahamas. Durant cette période, j'ai continué à collaborer avec Isaure sur les patrons de co-occurrence des ectoparasites du mullet.

A l'issue de cette expérience, j'ai suivi Scott France à l'Université de Louisiane à Lafayette, pour travailler sur l'écologie évolutive des coraux de grande profondeur. Une fois encore (c'est en fait typique aux États-Unis), j'ai construit mon sujet de thèse, et je l'ai partiellement financé. J'ai notamment obtenu, en première année de doctorat, une bourse de l'American Museum of Natural History de New York afin de passer un mois à Paris pour explorer les collections de coraux du Muséum national d'Histoire Naturelle. Lors de cette visite, j'ai rencontré Sarah Samadi, avec qui je collabore encore activement aujourd'hui. Mon travail avec Sarah a été, je pense, décisif pour ma carrière, car il m'a permis de publier plusieurs articles dans des revues de haut rang, et de développer un programme de recherche sur la délimitation des espèces et la taxinomie intégrative.

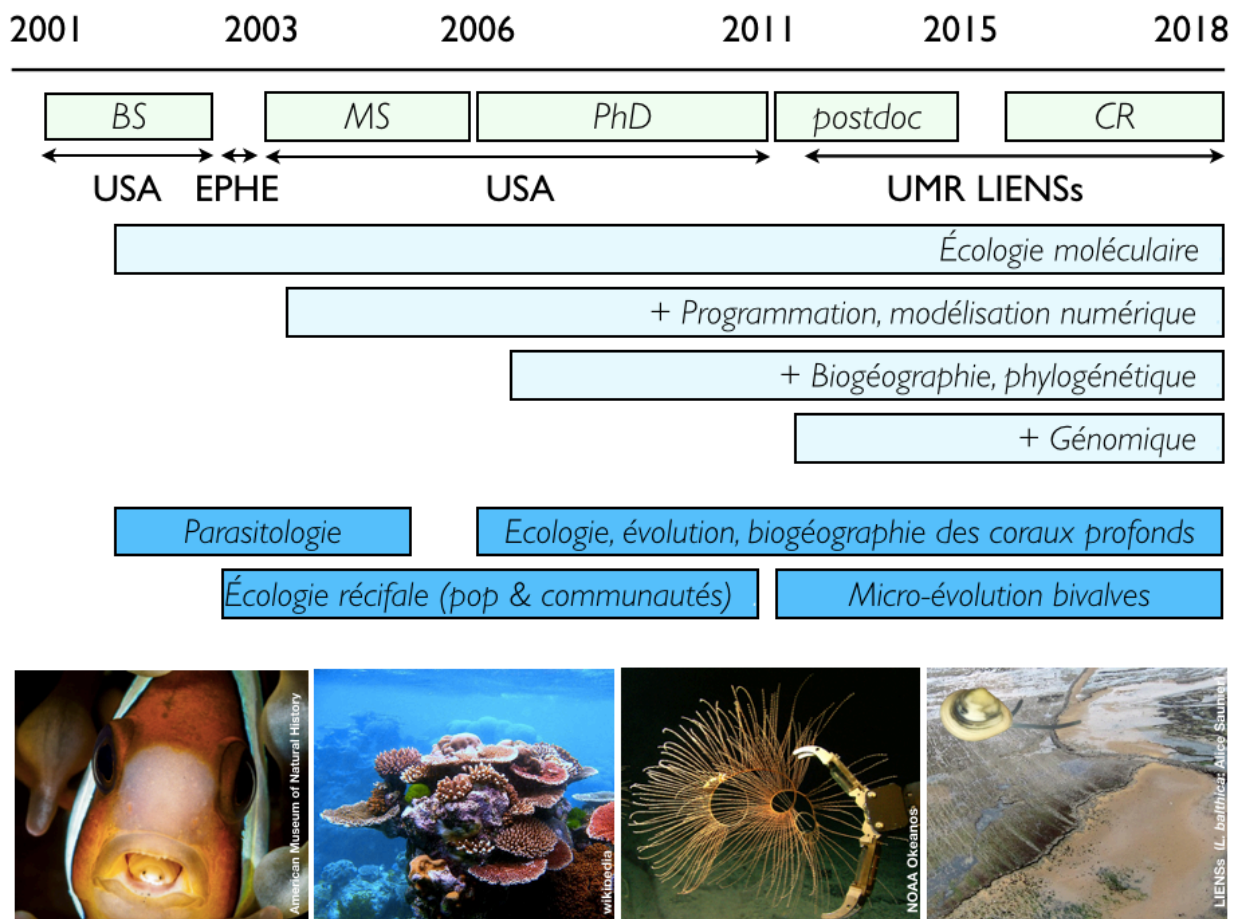
En résumé, ma mobilité aux États-Unis m'a donné l'opportunité de multiplier les expériences de recherche en travaillant sur divers modèles biologiques aussi bien terrestres (coléoptères), estuariens (acanthocéphales et copépodes parasites de poissons), que marins (coraux scléactiniaires, octocoraux, échinodermes et mollusques), toujours sur le thème central de la structure spatio-temporelle des populations, espèces et communautés, et son rôle dans la création et le maintien de la biodiversité. La flexibilité du système universitaire américain m'a permis de cumuler les expériences de terrain, de construire mes projets de thèse (masters et doctorat), d'obtenir mes propres financements de recherche, et de démarrer des collaborations nationales et internationales. Ce parcours universitaire atypique m'a donc permis de développer mon esprit d'initiative, d'acquérir une forte autonomie, tout en valorisant le travail collaboratif.

A l'issue de mon doctorat dans le laboratoire de Scott, en 2011, je souhaitais continuer mon travail sur la caractérisation de la biodiversité en milieu marin profond. Mes demandes de financement (e.g. programme Marie-Curie pour développer l'utilisation du

séquençage nouvelle-génération pour délimiter les espèces, en collaboration avec le MNHN de Paris) n'ayant pas abouti. une offre de post-doctorat au laboratoire LIENSs de l'Université de La Rochelle, postée par Pascale Garcia, a retenu mon attention. Le projet se proposait de rechercher les bases génétiques de l'adaptation locale chez le bivalve marin *Limecola balthica* (alors appelé *Macoma balthica*) à l'aide de données transcriptomiques. Ce sujet représentait un grand écart thématique: nouveau modèle biologique (les mollusques,), nouvelles échelles de temps et d'espace, nouveaux outils. Comme je le détaille plus tard dans la section "travaux antérieurs", cette nouvelle direction dans ma carrière scientifique m'a conduit à aborder de nouvelles questions, pour lesquelles les coraux de grandes profondeurs ne constituent pas un modèle très adapté.

Entre 2011 et 2015, j'ai donc travaillé au LIENSs en tant que post-doc. L'idée de Pascale était de préparer un candidat au concours CNRS, afin de renforcer la recherche en écologie évolutive au sein du LIENSs. Après quatre tentatives au concours CR2, j'ai été recruté en section 29 pour travailler sur la nature des barrières aux flux de gènes en milieu marin, en me focalisant sur le modèle *L. balthica*. Je maintiens cependant des collaborations actives avec le MNHN (voir section Collaborations).

Figure 1. Schéma résumant mon parcours professionnel. Boîtes vert clair: formations; bleu clair: compétences; bleu foncé: thématiques de recherche.

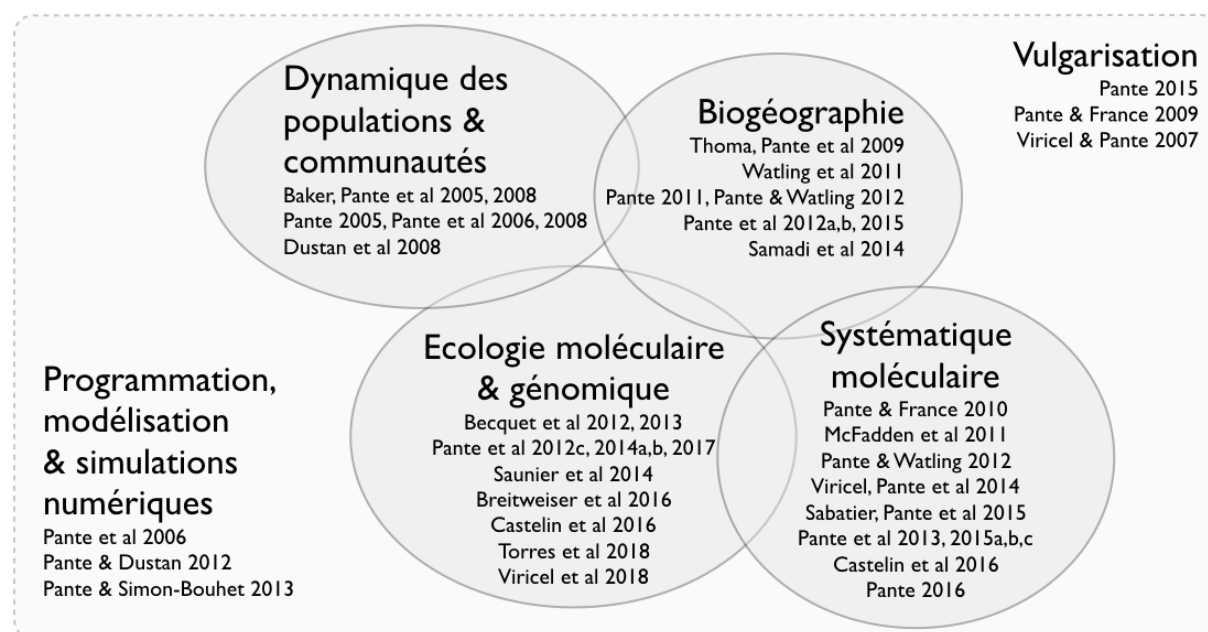


2. Thématiques de recherche

Mon activité de recherche¹ s'organise autour des questions suivantes : (1) Quels sont les paramètres qui dictent la répartition spatio-temporelle des individus, populations, et espèces ? (2) Comment ces paramètres influencent-ils les processus éco-évolutifs qui génèrent et maintiennent la biodiversité en milieu marin?

Durant mon parcours, j'ai pu multiplier les modèles d'études (e.g. parasites de poissons, coraux, échinodermes, mollusques, et mammifères marins), les approches (e.g. écologie descriptive et expérimentale, modélisation, génétique), et les échelles (de temps et d'espace) pour travailler sur ces questions (Figure 2).

Figure 2. Résumé de mes domaines de compétence, et publications associées.



L'essentiel de mon activité se divise en quatre thèmes (par ordre chronologique):

- l'écologie des populations de parasites,
- l'écologie des populations et communautés en milieu récifal,
- l'écologie, évolution, et biogéographie des coraux de grande profondeur, et
- les mécanismes micro-évolutifs qui génèrent et maintiennent les zones hybrides.

Dans la partie "Travaux antérieurs" de ce mémoire, je décris brièvement les trois premiers thèmes, sans entrer dans les détails car je ne travaille maintenant plus (a et b) ou peu (c) sur ces sujets (ils font, cependant, chacun l'objet de publications dans des revues de rang A). Je détaille également la transition entre les thématiques (c) et (d). La partie (d), qui représente maintenant le coeur de mon activité, est détaillée en partie 4 ("Programme de recherche"). Elle comprend aussi bien les projets aboutis que les projets en cours et futurs.

¹ Les références complètes pour les articles que j'ai co-signé sont disponible dans mon CV, en Annexe 1. Toutes autres références sont dans la section "Références".

3. Travaux antérieurs

a) Écologie des populations de parasites

Mes travaux sur les parasites, que j'ai menés en tant qu'étudiant au College of Charleston (B.S. et M.S., 2001-2005), sont axés sur la dynamique des populations selon les caractéristiques de l'hôte (âge, sexe), et aux fluctuations environnementales spatiales (e.g. gradient de salinité dans l'estuaire de Charleston) et saisonnières (Baker et al 2008), ainsi que sur la compétition intra- et inter-spécifique (Baker et al 2005). Ces études portent principalement sur le parasite polyopisthocotylide *Metamicrocotyla macracantha*, qui infecte les branchies du mulot *Mugil cephalus*. Le suivi saisonnier des populations de *M. macracantha* dans les estuaires de Caroline du Sud (USA) est en limite de nord de l'aire de répartition de l'espèce; les populations échantillonnées ne semblant pas compléter leur cycle de vie, celles-ci constituent probablement des populations puits.

Production : 2 articles de rang A
3 contributions à des congrès.

Expérience de terrain: pêche électrique et « trammel netting » dans les estuaires de la Caroline du Sud (USA).

b) Écologie des populations et communautés en milieu récifal

Mes travaux sur les invertébrés benthiques des récifs coralliens portent sur l'importance des paramètres environnementaux dans la structuration des communautés et des populations, tant à des échelles spatiales que temporelles (Pante et al 2006, 2008; Dustan et al 2008, Brumbaugh et al 2014). Le sujet de ma thèse de Masters of Science (College of Charleston, USA) porte sur le déclin d'un récif aux Bahamas et ses causes. Rainbow Gardens Reef a perdu, entre 1991 et 2004, près de 80% de sa couverture corallienne. L'étude de la structure de cette communauté, des conditions phénotypiques des coraux scléractiniaires, ainsi que des bandes de croissance de l'espèce constructrice *Orbicella annularis*, suggère que les actions couplées du blanchissement, de la bioérosion, et de tempêtes ont précipité le déclin de ce site.

Suite à mes travaux à Rainbow Gardens Reef, je me suis intéressé aux problèmes méthodologiques liés au suivi écologique des écosystèmes benthiques à l'aide de simulations numériques (Pante et Dustan 2012). Une des méthodes les plus employées pour le monitoring de ressources benthiques est l'estimation de la couverture corallienne à partir de vidéos et photos prises le long de transects. Dans Pante et Dustan (2012), j'ai évalué la robustesse de différentes stratégies d'échantillonnage, à l'aide de modélisations et simulations. Ces résultats ont permis la publication de préconisations sur la mise en œuvre de monitoring des récifs coralliens employant des transects vidéos. Ces conseils ont été adoptés par l'entreprise australienne « GeoOceans », qui a pour but l'estimation d'impacts environnementaux en milieu marin.

Production : 4 articles de rang A, 1 chapitre de livre, 1 rapport technique
8 contributions à des congrès internationaux.

Expérience de terrain: ~ 150 plongées en milieu tropical: Floride, Iles Cayman, Bahamas, Madagascar, Papouasie Nouvelle-Guinée.

Boîte 1 - Développement méthodologique: Importer, cartographier et analyser des données bathymétriques dans R

Au cours de mes projets de recherche, notamment lors de mon doctorat, j'ai été amené à développer de nouveaux outils d'analyses de données spatiales à l'aide de la plateforme de programmation et d'analyse statistique R (Pante et Dustan 2012; Pante & Simon-Bouhet 2013). Depuis 2012, je travaille en collaboration avec Benoit Simon-Bouhet (MCF, Université de La Rochelle) à la diffusion de ces outils sous la forme d'un paquet dédié, appelé *marmap*.

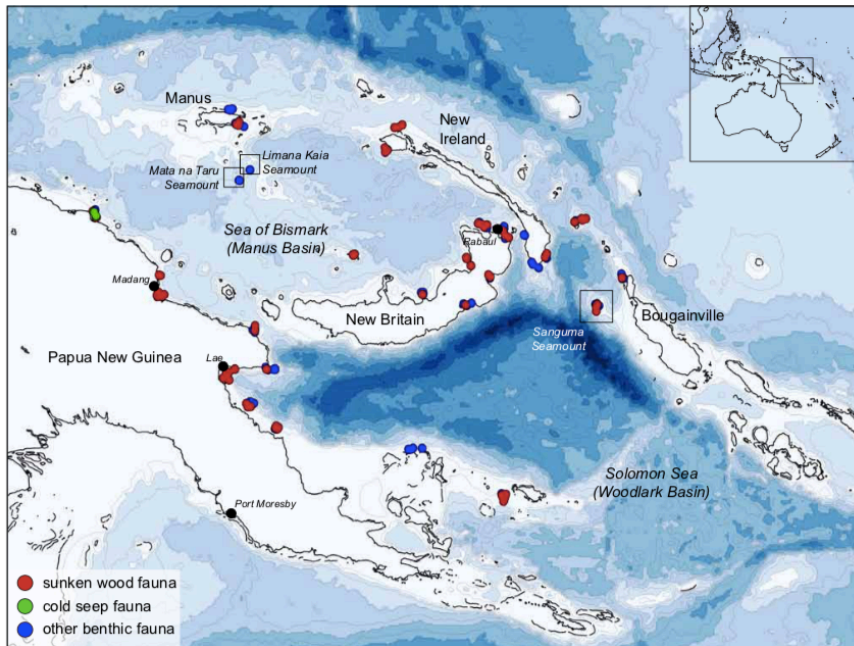


Figure 1. Map of Papua New Guinea and its satellite islands. Dots, color-coded based on faunal type, represent sampling stations. The inset provides a regional map. A marine geological map of PNG can be found in Tregoning et al. (2000).

Les outils contenus dans *marmap* ont pour objectifs la préparation de cartes bathymétriques et topographiques, de cartes d'échantillonnage pour l'écologie et l'évolution, l'analyse de données bathymétriques et topographiques (e.g. calculs de transects 2D et 3D, recherche de trajectoires contraintes par des lignes de contours, calcul de surfaces projetées, etc). Une partie importante de *marmap* consiste à

fournir des outils pour l'importation, l'exportation et la manipulation de données bathymétriques et topographiques (voir figure ; Pante et al 2012b).

Un article a été publié dans PLoS ONE (Pante & Simon-Bouhet 2013) pour détailler les caractéristiques de *marmap*. Cet article a été visionné plus de 19,000 fois et cité 88 fois sur Scopus (125 sur Google Scholar) depuis sa parution le 3 sept. 2013. Il était le 33ème article le plus visionné sur le site de PLoS ONE deux mois après sa mise en ligne. Le paquet *marmap* a été téléchargé plus de 44,500 fois depuis le site RStudio.

CRAN <https://cran.r-project.org/web/packages/marmap/index.html>

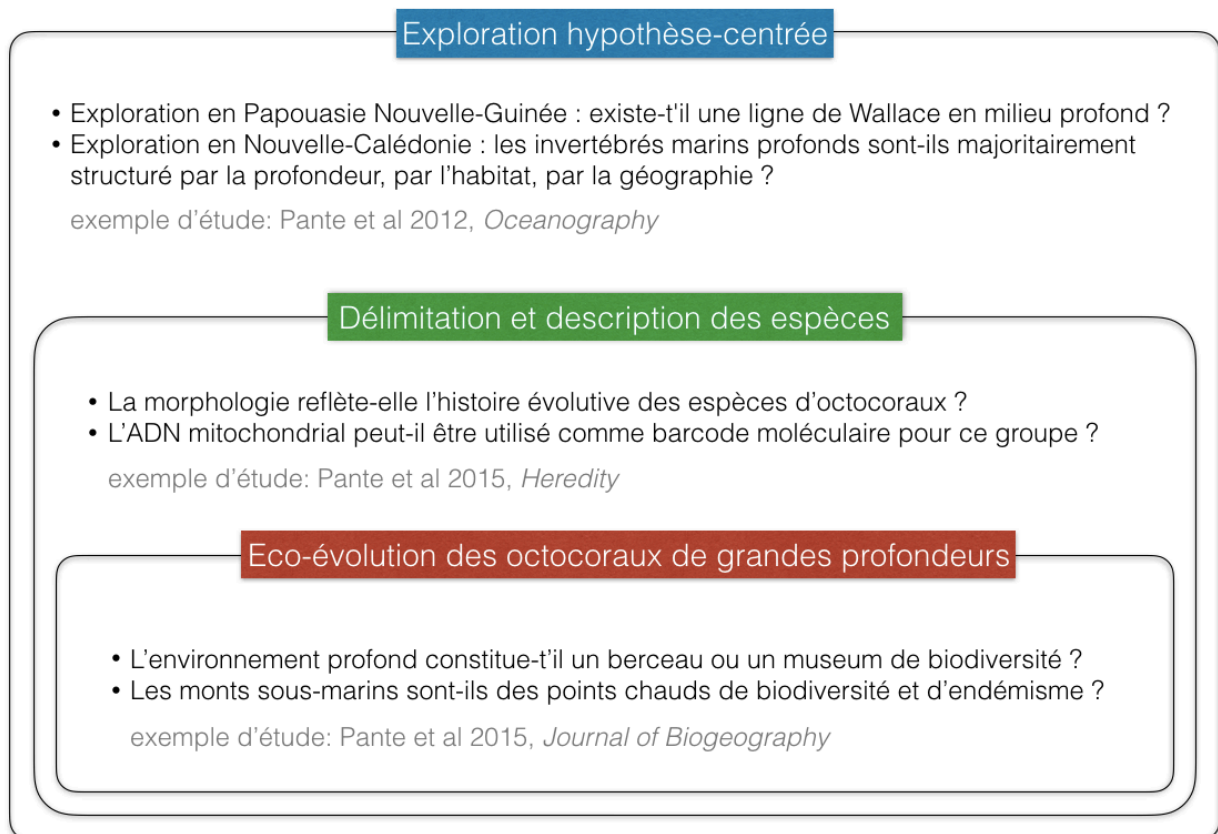
GitHub: <https://github.com/ericpante/marmap>

site dédié: <https://epante.wordpress.com/marmap/>

c) Biogéographie des coraux de grandes profondeurs

Pendant ma thèse de doctorat (Ph.D.), je me suis intéressé à l'origine de la biodiversité dans les zones de grandes profondeurs (> 200 m), particulièrement celle des coraux (Watling et al 2011). Mon travail s'est divisé en plusieurs chantiers complémentaires: (1) l'exploration des fonds marins (Pante et France 2009, Pante et al, 2012b), (2) la délimitation et la description de nouveaux taxa (Pante et France 2010, Pante et Watling 2012, McFadden et al 2011, Pante et al 2013, Pante 2016), et (3) l'utilisation de ces données pour mieux comprendre les mécanismes éco-évolutifs qui génèrent et maintiennent la biodiversité (Thoma et al 2009, Pante et al 2012c, Samadi et al 2014, Pante 2015abc). Les deux premiers chantiers permettent de construire le cadre de travail sur lequel viennent s'appuyer nos études sur la biologie évolutive et l'écologie des coraux de grandes profondeurs. Je détaille ci-dessous mes travaux sur l'évaluation de l'endémisme sur les monts sous-marins (Figure 3).

Figure 3. Grandes thématiques abordées dans le cadre de mes travaux sur les coraux de grandes profondeurs. Les articles cités sont disponibles en Annexe 2.



Les monts sous-marins constituent un laboratoire naturel pour l'étude de l'endémisme en milieu océanique

Les monts sous-marins sont des reliefs qui s'élèvent du plancher océanique sans atteindre la surface. Ces monts sont de véritables "laboratoires naturels" pour l'étude de la biogéographie et de l'endémisme de la faune benthique profonde (e.g. Shank 2010). En raison de leur origine volcanique, ces monts sont composés de substrats durs, pentus, et donc relativement peu recouverts par les sédiments. Ces caractéristiques contrastent avec les conditions trouvées dans les vallées et bassins océaniques environnants, couverts par

une épaisse couche de fines particules sédimentaires. Les monts sous-marins sont donc de véritables oasis basaltiques dispersées sur de vastes étendues sédimentaires. La différence entre bassins et monts ne se limite pas aux caractéristiques du substrat : lorsque les courants de fond rencontrent les monts, ils sont déviés et amplifiés. Ces courants enveloppent la surface des monts et empêchent les sédiments de se déposer en masse. Ils peuvent également former des “colonnes de Taylor”, masses d’eau qui tourbillonnent autour du mont et pourraient emprisonner la faune mobile (e.g. Mullineaux et Mills, 1997).

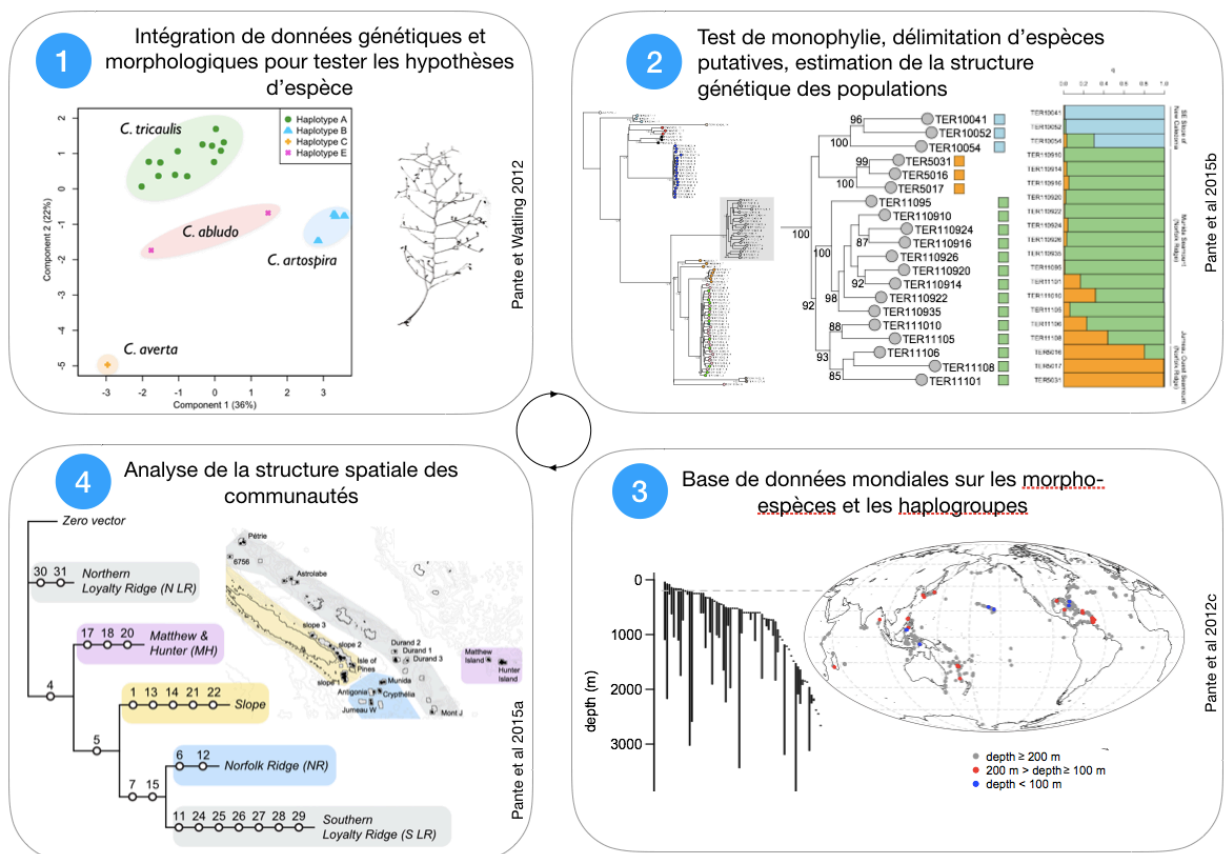
L’ensemble de ces caractéristiques géographiques, géologiques et océanographiques (isolement géographique entre monts, nature du substrat, courants...) a conduit les scientifiques à postuler que la faune des monts sous-marins pourrait être hautement endémique, et en effet, plusieurs études ont décrit un fort taux de restriction géographique de cette faune, notamment sur les monts du Pacifique sud-ouest (e.g. Richer de Forges et al. 2000). Cependant, les premières études de l’endémisme de la faune des monts sous-marins étaient limitées à une caractérisation de la biodiversité basée sur des critères morphologiques. De nouvelles études, basées sur des marqueurs génétiques, remettent en cause ces premiers résultats (e.g. Smith et al. 2004, Samadi et al. 2006, Thoma et al. 2009), et dévoilent la grande capacité de dispersion de nombreuses espèces typiques des monts sous-marins. Cependant, l’étude de l’endémisme et la connectivité entre monts et pentes océaniques souffre de plusieurs limitations (McClain 2007, Stocks et Hart 2007), notamment un important biais d’échantillonnage au niveau géographique, un manque d’intégration des données morphologiques et génétiques dans l’estimation de la biodiversité, et l’absence de “systèmes modèles” pour étudier ces questions de façon cohérente, à différentes échelles de temps et d’espace.

Le genre Chrysogorgia: développement d’un système modèle pour l’étude de l’endémisme des coraux sur les monts sous-marins

Dans le cadre de ma thèse, j’ai testé la pertinence du genre *Chrysogorgia* (Cnidaria: Anthozoa: Octocorallia: Chrysogorgiidae) pour étudier l’isolement de la faune des monts sous-marins (Figure 4). *Chrysogorgia*, avec environ 60 espèces décrites, est l’un des genres d’octocoralliaires les plus diversifiés, et possède une distribution bathymétrique et géographique remarquable, puisqu’on le trouve entre 10 et 4200 mètres de profondeur, à toutes les latitudes et dans tous les océans (Watling et al 2011). *Chrysogorgia* a été trouvé en abondance sur des monts sous-marins, ainsi que sur des pentes océaniques et continentales. Un de mes premiers chantiers a été de tester la monophylie du genre, à l’aide de marqueurs mitochondriaux et nucléaires (Pante et al 2012c). J’ai ensuite testé la congruence entre la variabilité morphologique et génétique. La structuration génétique au marqueur mitochondrial *msh1* correspond à l’alpha taxinomie inférée à partir de la morphologie des colonies (e.g. mode de branchement) et des polypes (e.g. composition et donation des sclérites dans les polypes; Pante et Watling 2012). Cette congruence entre variabilité morphologique et génétique permettrait de délimiter les espèces en se basant sur les haplotypes mitochondriaux. Ceux-ci, cependant, sont très peu divergents, une seule mutation sur le marqueur *msh1* séparant, a priori, différentes espèces de *Chrysogorgia*. L’ADN mitochondrial étant fortement conservé au sein des octocoraux, le barcoding moléculaire basé uniquement sur ce type de marqueurs reste peu résolutif

(McFadden et al. 2010). En effet, des espèces ayant divergé récemment peuvent être caractérisées par des haplotypes mitochondriaux identiques. Afin de tester la résolution des marqueurs mitochondriaux pour le barcoding moléculaire, nous avons entrepris de tester nos délimitations basées sur les haplotypes *msh1* à l'aide de données de type RAD-seq (Pante et al 2015b). Cette étude suggère qu'environ 70% des espèces sont correctement délimitées avec les marqueurs mitochondriaux (Figure 4). Lorsque la délimitation basée sur l'ADN mitochondrial est utilisée pour inférer la structure des communautés (voir ci dessous), ce taux d'erreur reste acceptable, et entraîne des biais modérés sur la mesure de l'endémisme local (Pante et al 2015a).

Figure 4. Développement du genre *Chrysogorgia* comme modèle d'étude de la biogéographie des organismes benthiques profonds. Taxinomie traditionnelle et données morphologiques sont confrontés (1); les hypothèses d'espèces sont testés avec un grand nombre de marqueurs (2); les unités taxinomiques sont ensuite utilisées pour adresser des questions éco-évolutives (3-4). L'échantillonnage géographique et moléculaire s'améliorant, les hypothèses d'espèces sont affinées (boucle centrale).



Les campagnes “Tropical Deep-sea Benthos” et l’exploration de la biodiversité de la faune benthique profonde du Pacifique sud-ouest.

Depuis environ 25 ans, le Muséum national d'Histoire naturelle (MNHN) et l'Institut de Recherche pour le Développement (IRD) organisent des campagnes océanographiques visant à décrire la faune benthique profonde du Pacifique sud-ouest entre 100 et 1500 mètres de profondeur. Ce programme de recherche, appelé “Tropical Deep-sea Benthos” (TDSB), unique en son genre, est le plus complet des efforts d'échantillonnage

de la faune benthique profonde de l'Indo-Pacifique (Bouchet et al. 2008). Depuis le début de ces campagnes, plus de 3000 stations ont été échantillonnées, et plus de 2000 nouvelles espèces ont été décrites. Au-delà de sa mission d'exploration, le programme TDSB alimente plusieurs thématiques de recherche, dont la description des mécanismes associés à la diversification de la faune benthique des monts sous-marins, et le rôle de l'isolement géographique dans la diversification de cette faune.

Au cours des campagnes TDSB notamment "Terrasses", "ExBoDi", "KanaDeep" (Nouvelle-Calédonie), "BioPapua" et "Madang" (Papouasie Nouvelle-Guinée), et "BioMaGlo" (Mayotte, Glorieuses) auxquelles j'ai pu participer, un grand nombre de colonies de *Chrysogorgia* a été échantillonné. J'ai ainsi pu récolter près d'un millier de colonies de *Chrysogorgia*. Ces spécimens, avec ceux collectés précédemment dans le Pacifique et l'Atlantique, constituent une collection unique pour étudier le degré d'endémisme sur les monts sous-marins ainsi que les mécanismes qui génèrent et maintiennent cette restriction géographique. *Chrysogorgia* ne semble montrer aucun signe d'endémisme sur les monts de l'Atlantique sud-ouest (Thoma, Pante et al. 2009). En effet, les haplotypes caractérisés par une distribution géographique restreinte sont aussi les plus rares (ou les moins bien échantillonnés). En revanche, la répartition géographique des espèces du Pacifique sud-ouest paraît être beaucoup plus restreinte (Pante et al 2015a). Cette différence semble partiellement due aux différences de régimes bathymétriques observées entre les monts sous-marins Atlantiques (1500-2000 m) et Pacifiques (70-1200 m). Cette hypothèse, cependant, ne pourra être confirmée sans un échantillonnage plus profond de la zone de travail Pacifique.

Production : 14 articles de rang A, un manuscrit en préparation

1 article pour le grand public, 1 chapitre de livre, 1 article de magazine

15 contributions à des congrès et 7 séminaires.

Expérience de terrain: participation à 7 campagnes océanographiques (Atlantique, Pacifique et Antarctique); utilisation de robots télé-opérés (ROV), DropCam, TowCam, dragage et chalutage. Plus de 140 jours passés en haute mer.

d) De la macro-évolution des coraux à la micro-évolution des mollusques

Bien que mon travail de doctorat sur les coraux de grandes profondeurs ait amené de nombreuses questions très intéressantes sur le fonctionnement de ce milieu (par exemple, tempo de l'évolution et de la spéciation par rapport aux zones littorales de faible profondeur ; phénomènes « d'émergence » de lignées évolutives de zones profondes aux zones littorales peu profondes), ces coraux constituent néanmoins un modèle d'étude sur lequel il est toujours très difficile de travailler.

Premièrement, ces organismes sont difficiles à échantillonner, ce qui limite grandement le nombre de spécimens disponibles pour les études sur la génétique des populations. En effet, les campagnes d'échantillonnage sont très onéreuses et mobilisent beaucoup de moyens. De plus, la collecte s'associe très souvent à une dégradation de l'ADN (e.g. choc thermique lors de la remontée d'un chalut de 1000 m de profondeur où la température est de 4°C, vers les eaux de surfaces qui peuvent atteindre 30°C). Deuxièmement, les coraux sont dotés de génomes mitochondriaux et nucléaires complexes et peu variables

(particulièrement le génome mitochondrial), ce qui complique également le travail génétique à l'échelle des populations.

À cause de ces verrous, les coraux de grandes profondeurs ne constituent pas un modèle de choix pour l'étude des processus évolutifs dans le milieu marin en général. Un bon modèle doit être caractérisé par des populations de grande taille, des niveaux de variabilité génétique qui facilitent l'étude des populations, et facilement accessible à la collecte. Les bivalves marins, particulièrement les espèces intertidales, *Limecola balthica*, à l'instar des coraux de grandes profondeurs, s'inscrivent dans cette description.

En 2011, j'ai initié un post-doctorat sur la génétique des populations nord-est atlantiques de *Limecola balthica*, afin d'étudier la structure génétique chez cette espèce le long d'un gradient latitudinal. L'objectif principal de ce projet était de rechercher à l'aide de données transcriptomiques (et donc, *a priori*, non neutres), de part et d'autre de deux barrières génétiques, des gènes impliqués dans l'adaptation locale, de part et d'autre de deux barrières aux flux de gènes. Dans la seconde partie, j'expose les résultats de cette étude, ainsi que les questionnements qui en découlent.

Production : 5 articles de rang A , 1 manuscrit soumis, plusieurs manuscrits en préparation
18 contributions à des congrès et 4 séminaires.

Expérience de terrain: échantillonnage le long des côtes françaises et nord américaines.

4. Programme de recherche

Dynamique spatio-temporelle des zones hybrides marines: nature des barrières aux flux de gènes et implications pour le potentiel adaptatif

a) Introduction

1. Contexte: changements climatiques et impact sur la biodiversité

La biosphère est aujourd'hui de plus en plus affectée par la destruction et la fragmentation de l'habitat, la surexploitation, et l'introduction d'espèces invasives (e.g. Groom et al 2006). Ces pressions, qui touchent aussi bien les écosystèmes aquatiques que terrestres, sont exacerbées par un réchauffement climatique global (e.g. Bell et Collins 2008). La température moyenne de surface de la terre a augmenté de 1°C (Berkeley Earth research group 2011) entre 1980 et 2010, et la température moyenne des eaux de surface de l'Atlantique nord-est a augmenté de 0,23°C par décennie entre 1977 et 2007 (e.g. Goikoetxea et al 2009). Le processus s'accompagne de profondes mutations phénologiques et biogéographiques. Ainsi, à partir d'une méta-analyse sur plus de 1700 espèces, Parmesan et Yohe (2003) ont montré un changement des aires de répartition cohérent avec la réponse attendue au réchauffement climatique: déplacement vers les pôles, vers des altitudes plus élevées pour les espèces terrestres ou de plus grandes profondeurs pour les espèces aquatiques (voir aussi Dulvy et al 2008 ; Wetthey et Woodin 2008).

Le littoral, interface entre terre et mer, est particulièrement touché par ces changements climatiques, puisqu'il subit à la fois les pressions environnementales terrestres et marines. C'est au sein des écosystèmes littoraux que les mouvements de populations vers de plus hautes latitudes sont particulièrement importants, avec une moyenne de 50 Km par décennie, soit davantage que la plupart des variations de limite d'aire de répartition enregistrées pour les organismes terrestres (Parmesan et Galbraith 2004 ; Helmutt et al 2006 ; Poloczanska et al 2013). Sur la côte Atlantique nord-est, notamment, la moule commune *Mytilus edulis* et la telline *Limecola balthica* auraient migré respectivement de 1000 et 300 Km vers le Nord en moins de 30 ans (Salvigsen et al 1992 ; Weslawski et al 1997 ; Berge et al 2005 ; Jansen et al 2007a).

Or, alors que ces événements de rétraction ou de déplacement d'aire de répartition sont susceptibles d'avoir des conséquences graves sur le fonctionnement et la structure des écosystèmes, surtout lorsque des espèces clés sont concernées, la réponse évolutive des populations situées en limite d'aire aux pressions environnementales reste mal connue, voire peu étudiée (Walther et al 2002 ; Parmesan et Yohe 2003). Décrire et comprendre les altérations génétiques des organismes face aux changements climatiques représente donc un défi d'envergure pour les biologistes de l'évolution (Bell et Collins 2008 ; Bell et Gonzales 2009).

2. Aire de répartition, adaptation, et hybridation

Afin de mieux comprendre la réponse évolutive des espèces marines aux changements climatiques, je propose d'étudier la dynamique des populations limitrophes (c'est-à-dire situées près de la limite de leur aire de distribution) et leur adaptation à des conditions environnementales locales changeantes.

Ces populations possèdent des caractéristiques démographiques et génétiques qui diminuent leur capacité d'adaptation (Eckert et al 2008). Le long d'un gradient environnemental, elles sont en équilibre instable entre mal-adaptation aux conditions locales et déclin démographique, avec des performances physiologiques médiocres et un taux de croissance réduit (Brown et al 1995 ; Thomas et Kunin 1999). Par conséquent, les organismes limitrophes forment souvent de petites populations fragmentées, vulnérables à la dérive génétique et exposées à l'effet Allee (Courchamp et al 1999 ; Alleaume-Benharira et al 2006). Cette fragmentation est susceptible d'être exacerbée dans le contexte du réchauffement climatique puisque les températures élevées peuvent diminuer la durée de vie larvaire (Hoegh-Guldberg et Pearse 1995) et le succès du recrutement (e.g. Pineda et al 2002).

La vitesse des changements environnementaux est d'une importance fondamentale, car elle est la principale limitation à l'élaboration d'un processus adaptatif. En effet, la différenciation adaptative des populations limitrophes ne peut se mettre en place que si les effets diversifiants de la sélection naturelle surpassent ceux de la dérive génétique et des échanges migratoires. Le flux de gènes provenant de populations centrales vers les populations périphériques freine la mise en place d'adaptations locales. Une forte connectivité entre populations peut donc mener à une mal-adaptation chronique des populations limitrophes, à moins qu'elle ne soit équilibrée par d'importantes pressions de sélection (Ronce et Kirkpatrick 2001 ; Bolnick et al 2008). Pour autant, chez les organismes marins présentant des paramètres démographiques extrêmes (très grande fécondité, très grande taille des populations) et souvent capables de dispersion à longue distance par une phase larvaire planctonique, les polymorphismes d'adaptation aux variations locales de leur environnement sont maintenus. La compréhension de ce paradoxe est fondamentale pour appréhender les mécanismes de l'adaptation contemporaine des espèces marines, notamment face aux changements environnementaux rapides engendrés par les pressions anthropiques tels que les changements climatiques et la dégradation des milieux (pollutions).

Les zones hybrides constituent un laboratoire naturel pour étudier la mise en place d'adaptations aux conditions environnementales locales, car elles marquent la séparation spatiale entre des stocks génétiques différenciés qui se mélangent peu. Elles peuvent être maintenues par une multitude de facteurs environnementaux et/ou intrinsèques (e.g. Barberousse et al 2010), de l'isolement écologique (barrière au flux de gène de type exogène, fortement dépendante de l'environnement) à l'incompatibilité génétique (barrière intrinsèque de type endogène). Certains gènes peuvent ainsi contribuer à limiter le mélange entre pools géniques différenciés, parce qu'ils contribuent à l'adaptation aux conditions environnementales locales (sélection locale ; e.g. Nosil et al 2009), ou parce qu'ils sont incompatibles avec les gènes des autres pools géniques (sélection contre les hybrides ; synthèses de Burton et Barreto 2012 ; Burton et al 2013). Ces différents types de barrières au flux de gènes peuvent se coupler et ainsi renforcer la divergence entre populations (Barton 1979 ; Bierne et al 2011 ; Abbott et al 2013). Les interactions entre les barrières intrinsèques et l'environnement, ainsi que l'impact de ces interactions sur les processus adaptatifs sont encore très mal connus et demandent à

être étudiés plus en détail (e.g. Nosil 2012, David et Troussellier 2013). L'hybridation et l'introggression panachent des génomes différenciés, susceptibles de conduire à de nouvelles combinaisons génétiques et entraîner de nouvelles adaptations (e.g. Stebbins 1959, Mallet 2005, Schwenk et al. 2008, Nolte et Tautz 2010). Ces phénomènes de mélanges contribuent non seulement à maintenir le stock de variation génétique, mais peuvent également produire de nouveaux caractères ou des formes extrêmes de caractères existants (« ségrégation transgressive » ; Rieseberg et al 1999, 2003). L'hybridation pourrait ainsi promouvoir le phénomène de radiation adaptative en permettant l'utilisation de nouvelles niches écologiques (Seehausen 2004).

Les zones hybrides peuvent être nombreuses en milieu marin, particulièrement dans la zone littorale, dont la biogéographie est fortement influencée par cycles de glaciations et déglaciation qui ont pour impact de séparer puis remettre en contact populations et espèces (e.g. Maggs et al 2008). Leur étude dans le contexte de la mise en place d'adaptation aux conditions environnementales est donc particulièrement pertinente chez les organismes marins, qui conjuguent souvent un fort flux génique, un polymorphisme important, et une structuration géographique faible (e.g. Bierne et al 2003).

3. Modèle d'étude : le cas de *Limecola balthica*

Une espèce sentinelle idéale :

La prédiction des effets des changements climatiques globaux est grandement facilitée par le suivi d'espèces dites sentinelles, organismes qui répondent rapidement aux variations environnementales. Une espèce sentinelle idéale doit (i) faire partie d'un groupe diversifié, (ii) avoir une fécondité élevée et un développement rapide, et (iii) avoir une réponse rapide et mesurable aux augmentations de température (Grosell et Walsh 2006). Les organismes de la zone intertidale constitueraient des espèces sentinelles des changements climatiques globaux pertinentes, du fait de l'influence couplée des variations environnementales terrestres et marines (e.g. Helmut et al 2006).

Dans ce contexte, *Limecola balthica* apparaît comme modèle d'étude très intéressant. Ce bivalve, qui peuple les milieux sablo vaseux intertidaux et subtidaux de l'ensemble des littoraux de l'hémisphère nord, occupe une place stratégique dans les réseaux trophiques littoraux. La grande quantité de données déjà disponibles sur la physiologie (e.g. Jansen et al 2007ab ; Beukema et al 2009 ; Van Colen et al 2012), l'écotoxicologie, (e.g. Sokolowski et al 1999 ; Duquesne et al 2004), l'écologie (e.g. Bouma et al 2001), la génétique des populations (e.g. Nikula et al 2007, 2008 ; Becquet et al 2012, 2013) et la génomique (e.g. Becquet 2011 ; Pante et al 2012a) de *L. balthica* renforce le potentiel de cette espèce comme modèle d'étude. En effet, une recherche Scopus avec les mots clés « *Limecola balthica* » ou « *Macoma balthica* » (le nom de genre a changé récemment; Huber et al 2015) produit 753 résultats (recherche 03/09/2018).

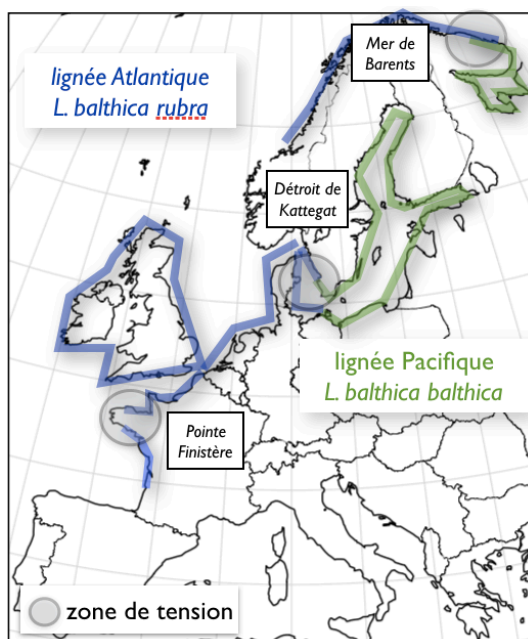
Limecola dans le contexte de réchauffement climatique :

Limecola balthica est peu tolérante aux augmentations de température, susceptibles d'impacter la production de gamètes, les migrations annuelles, le taux de croissance, la biomasse, et la survie des adultes (Philippart et al 2003, Beukema et al 2009). Cette espèce semble être d'ores et déjà affectée par les changements globaux contemporains. En effet, trente années de surveillance des populations ont révélé que

l'aire de répartition se décale rapidement vers le Nord parallèlement à l'élévation de la température des eaux de surface dans le golfe de Gascogne (e.g. Goikoetxea, 2009). Autrefois située en Galice (Espagne ; Otero et Millan 1972), la limite sud de l'aire de répartition de *L. balthica* se trouve désormais dans l'estuaire de la Gironde (France ; Bachelet 1980). Des expériences de transplantation de spécimens de *L. balthica* provenant de la Gironde dans l'aire de répartition d'origine ont montré que les températures estivales et automnales provoquent une surconsommation d'énergie menant à la diète, puis la mort des individus (Jansen et al 2007a). Les populations de *L. balthica* en limite sud de répartition subissent un décalage phénologique (déclenchement précoce de la ponte) de -1.7 jours par an depuis 1977 (Saunier, Pante et al soumis). Ce décalage est deux fois plus rapide que celui observé en Mer des Waddens pendant la même période (Philippart et al 2003).

La raréfaction, voire la disparition de ce bivalve dans les pertuis charentais, lieu de séjour hivernal de nombreuses espèces d'oiseaux limicoles, aurait potentiellement des conséquences dramatiques sur la faune avicole. En effet, *Limecola balthica* est une espèce clé du réseau trophique des littoraux européens (Philippart et al 2003). C'est une proie privilégiée des oiseaux migrateurs (Piersma et Beukema 1993), des macro-invertébrés (Edjung et Bonsdorff 1992) et des poissons (Mattila et Bonsdorff 1998). Les études menées au sein de notre laboratoire montrent son importance quantitative et qualitative dans le régime alimentaire du bécasseau maubèche (*Calidris canutus*) et de la barge à queue noire (*Limosa limosa*) (Quaintenne et al 2010; Robin, communication personnelle). Ainsi, la diminution des tailles de populations de *L. balthica* en mer des Waddens a impacté le réseau trophique de l'écosystème local. La succession d'hivers doux au cours des années 1988 à 1990 a limité le recrutement des bivalves (dont *L. balthica*), ce qui a entraîné la diète, l'émigration de masse, mais aussi la mort de nombreux oiseaux limicoles prédateurs de bivalves (Beukema et Cadée 1996 ; Camphuysen 2002).

Figure 5. Distribution contemporaine des lignées mitochondriales de *Limecola balthica* en Europe et position des zones de contacts entre lignées (Nikula et al 2007).



Limecola et l'étude de la dynamique des zones hybrides :

Limecola balthica est considéré comme étant parmi les meilleurs modèles d'étude des zones hybrides en milieu marin (Strelkov et al 2007 ; Riginos et Cunningham 2007). En effet, la colonisation épisodique de *L. balthica* a créé de nombreuses opportunités de remise en contact de différents stocks génétiques (admixture) et de mise en place de plusieurs zones hybrides en Atlantique (Nikula et al 2007; Figure 5). Deux sous espèces de *L. balthica* existent en Atlantique Nord : une lignée pacifique (*L. balthica balthica*) présente en mer Baltique et en mer Blanche, et une lignée atlantique (*L. balthica rubra*) présente en mer de Norvège, en mer du Nord et le long des côtes Britanniques (données allozymes : Väinölä 2003 ; ADN mitochondrial : Luttikhuisen et al 2003 ; Nikula et al 2008). Les zones de contact entre ces lignées coïncident avec des zones hybrides. En Europe, une zone de transition a été décrite dans le Détroit

de Kattegat, entre la Suède et le Danemark (Nikula et al 2007, 2008). La mer de Barents et la mer Blanche abritent, quant à elles, des mélanges génétiques typiques d'essaims d'hybrides, c'est-à-dire des zones où des hybrides féconds ont un avantage sélectif et persistent dans la population (Strelkov et al 2007).

Au Sud, dans l'estuaire de la Gironde, l'analyse de marqueurs mitochondriaux révèle une signature de variabilité mitochondriale unique, avec les haplotypes relativement divergents de ceux des autres populations européennes et pacifiques (Luttikhuisen et al 2003 ; Nikula et al 2008), signature non observée avec des marqueurs allozymiques (Hummel et al 1995, 1997 ; Strelkov et al 2007). Différents marqueurs génétiques ont donc montré deux tendances contrastées pour les populations dans le golfe de Gascogne: (i) une absence de différenciation allozymique entre populations nord et sud, couplée à une faible diversité génétique dans le Sud (résultat cohérent pour des populations situées près de la limite de répartition de l'espèce), et (ii) une différenciation mitochondriale et nucléaire significative entre populations nord et sud. Ces études étaient cependant limitées par un très petit nombre d'échantillons au sud de la Manche. Un échantillonnage à plus fine échelle mené dans le Golfe de Gascogne jusqu'au front de la limite de répartition de l'espèce et une analyse biogéographique basée sur un marqueur mitochondrial (*cox1*) et huit marqueurs microsatellites nucléaires confirment une rupture au flux de gènes dans le golfe de Gascogne, près de la Bretagne (Becquet 2011 ; Becquet et al 2012). Cependant, la position exacte de cette zone n'est pas encore connue. Egalement, l'étude reste limitée par le nombre de marqueurs génétiques et leur puissance statistique. L'utilisation de nouvelles technologies de séquençage de l'ADN a permis de multiplier le nombre de marqueurs, et de poursuivre l'étude des zones hybrides Finistère et Baltique à l'échelle du transcriptome (Pante et al 2012a ; détails plus bas).

L'exemple de *Limecola balthica* est donc particulièrement intéressant, puisqu'il regroupe différentes zones hybrides dont l'origine et la structure semblent être différentes :

- a. Les populations échantillonnées de part et d'autre de l'entrée de la Mer Baltique (Déroit de Kattegat) ont divergé il y a 2 à 3,5 millions d'années (lignées *rubra* et *balthica*, Nikula et al 2007). Cette ancienne divergence contraste avec une origine relativement récente de la Mer Baltique et de son peuplement par des espèces Atlantiques (il y a environ 7 500 ans ; Luttikhuisen et al 2003 ; Nikula et al 2007). Ce patron biogéographique pourrait être expliqué par (1) la remise en contact secondaire de populations issues d'événements différents de recolonisation du Pacifique vers l'Atlantique, puis (2) le couplage des zones de contacts secondaires avec des barrières environnementales (dans le Déroit de Kattegat, un gradient de salinité fort, et un faible mélange des masses d'eau qui défavorise la dispersion larvaire ; Bierne et al 2011). Une autre zone de tension entre lignées *rubra* et *balthica* est observée en Mer de Barents, et son origine pourrait être similaire à celle de Kattegat (Bierne et al 2011).
- b. Le long des côtes françaises, la lignée *rubra* est principalement divisée en deux clades, séparés par la pointe Finistère (Luttikhuisen et al 2003 ; Becquet et al 2012). Ils auraient divergé il y a au moins 110 000 ans (Luttikhuisen et al 2003). La zone de tension entre lignées *rubra* pourrait, quant à elle, être calée sur les changements environnementaux observés de part et d'autre de la pointe Finistère (changements de régimes thermiques, faible occurrence d'habitats favorables).

L'architecture complexe de ces zones hybrides est donc d'intérêt pour l'étude de la mise en place et du maintien des barrières aux flux de gènes, de leur nature (endogène

versus exogène), de leur interaction avec l'environnement, et enfin de leur rôle dans le processus adaptatif.

*L'utilisation de *Limecola balthica* en tant que modèle d'étude*

Le modèle *Limecola balthica* présente plusieurs avantages. Tout d'abord, ce bivalve est relativement facile à échantillonner (à pied, à marée basse en France) ; pour de nombreuses localités françaises, des cartes de densité de population existent (e.g. Bocher et al 2007). Egalement, il se démarque de la moule (espèces *Mytilus edulis*, *M. trossulus* et *M. galloprovincialis*), un autre groupe étudié dans le cadre de l'adaptation locale, de l'hybridation et des conséquences génétiques et démographiques des changements climatiques. *L. balthica* constitue en outre un groupe de populations naturelles non cultivées et les données actuelles ne montrent aucune indication que la structure génétique des populations atlantiques aient été affectée par l'activité humaine (par exemple, transport contemporain de larves dans les eaux de ballast ; Nikula et al 2007). Enfin, *L. balthica* et *Mytilus* sp. sont aussi représentatifs d'habitats différents (substrats sablo-vaseux et rocheux, respectivement).

b) Projets en cours et perspectives

Mon programme de recherche à moyen terme vise à décrire l'architecture et la dynamique temporelle des zones hybrides européennes, et plus particulièrement de la zone Finistère située à proximité de la limite d'aire de répartition de l'espèce, en intégrant les outils de la génomique aux méthodes de prédiction par modélisation et simulation. Ce programme a débuté en 2011 lors du démarrage de mon post-doc au LIENSs.

Axe 1 : Structuration des populations et nature des barrières aux flux de gènes

(i) Architecture géographique des zones hybrides

L'étude préliminaire de Becquet (2011), qui a mis en évidence une barrière au flux de gène entre les populations de *Limecola balthica* de part et d'autre de la pointe Finistère, a positionné cette espèce comme sentinelle des changements environnementaux du littoral français. Ce travail, basé sur un marqueur mitochondrial et huit marqueurs microsatellites, souffre cependant d'un manque de résolution spatiale et de puissance statistique. En effet, la capacité de détection de différences génétiques entre populations est directement liée au nombre de loci utilisés (e.g. Gaggiotti et al 1999), et seuls huit marqueurs nucléaires ont pu être développés par Becquet (2011).

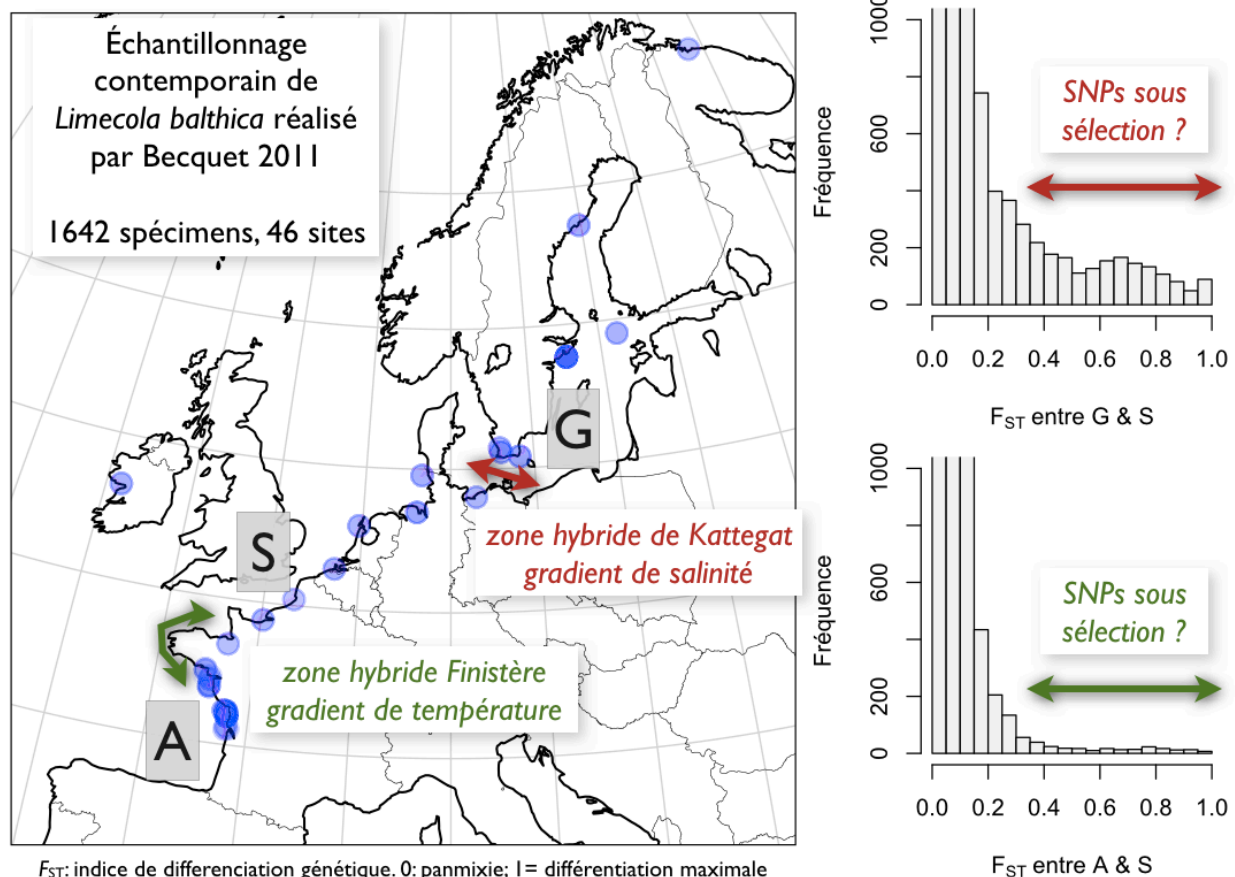
Les SNPs (Single Nucleotide Polymorphisms) sont des marqueurs de choix pour l'étude de la structuration des populations à une échelle spatiale fine. Extrêmement abondants dans le génome (e.g. Sauvage et al 2007), et notamment chez *Limecola balthica* (Pante et al 2012a), ils sont relativement faciles à génotyper puisque de très petits fragments d'ADN sont analysés, y compris sur des échantillons anciens au matériel génétique potentiellement dégradé (Morin et McCarthy 2007). Les SNPs sont des marqueurs bi-alléliques co-dominants au taux de mutation bas, ce qui limite les problèmes de forte variation génétique intragroupe. Les modèles de mutation de l'ADN sont simples, ce qui facilite (i) l'utilisation et l'interprétation de statistiques tels que les

indices de fixation de Wright (e.g. F_{ST}) (Meirmans et Hedrick 2011), (ii) la mise en place de projets de modélisation réalistes des variations génétiques au cours du temps, et (iii) les tentatives de prédiction de la trajectoire des populations de *Limecola balthica* (voir Objectif n°. 4). Les simulations de Morin et al (2009) ont ainsi montré que 30 SNPs permettent de détecter un niveau de différenciation modéré ($F_{ST} = 0.01$), alors que 80 SNPs permettent de mettre en évidence un niveau de différenciation très faible ($F_{ST} < 0.005$).

Dans le cas de *Limecola*, le scan génomique entrepris par Pante et al (2012a) a révélé plus de 17 000 SNPs présentant une très forte densité sur le génome. Ces SNPs ont été détectés à partir de 30 individus collectés sur trois localités différentes : le Pertuis Charentais en limite sud de l'aire de répartition (site « A » sur la Figure 6), la Baie de Somme (« S »), en aval de la zone de rupture au flux de gènes identifiée par Becquet (2011), et la mer Baltique (Baie de Gdansk ; site « G »), où réside la sous-espèce *Limecola balthica balthica*. L'analyse des fréquences alléliques de ces 17 000 SNPs pour 30 individus confirme la structure génétique hiérarchique forte inférée à partir des données mitochondriales et microsatellites.

A la suite de cette étude, notre objectif fut de préciser l'architecture de la zone hybride en utilisant un panel de SNPs choisis parmi cette banque de 17 000 marqueurs, pour un plus grand nombre de populations et d'individus (points bleus sur la carte ci dessous). Les technologies « GoldenGate » et « BeadXpress » d'Illumina ont été choisies à cette fin, car elles permettent le génotypage simultané de 384 marqueurs

Figure 6. Zones hybrides entre lignées évolutives de *Limecola balthica* en Europe, et résultats du séquençage 454 entre trois populations (A, G, S) [Pante et al 2012a].



SNP (e.g. Hubert et al 2010 ; Hoffman et al 2012). Ces données, en cours d'analyse, montrent une structure hiérarchique des populations aux sein de l'aire de répartition: (1) séparant les lignées rubra et balthica, (2) au sein de rubra, séparant les populations au sud de la Loire aux populations échantillonnées au nord de la Loire, (3) au sein de ces dernières, séparant les populations échantillonnées de part et d'autre de la pointe du Cotentin. Les prochaines étapes d'analyses viseront à caractériser, à l'aide des outils de la théorie des clines, l'architecture géographique des zones hybrides européennes (épicerie, largeur, asymétrie du flux de gènes ; e.g. Coustau et al 1991 ; Bierne et al 2003 ; Derryberry et al 2014).

(ii) Architecture génomique des zones hybrides

Cette première étape de cartographie fine des zones hybrides, quoique nécessaire, apporte peu d'éléments sur l'architecture génomique de la zone hybride ou de la nature des barrières aux flux de gènes. Des données sur l'identité des gènes impliqués dans la différenciation inter-populationnelle, les interactions épistatiques, et les interactions génome – environnement sont pourtant indispensables pour appréhender l'importance des zones hybrides dans le processus adaptatif.

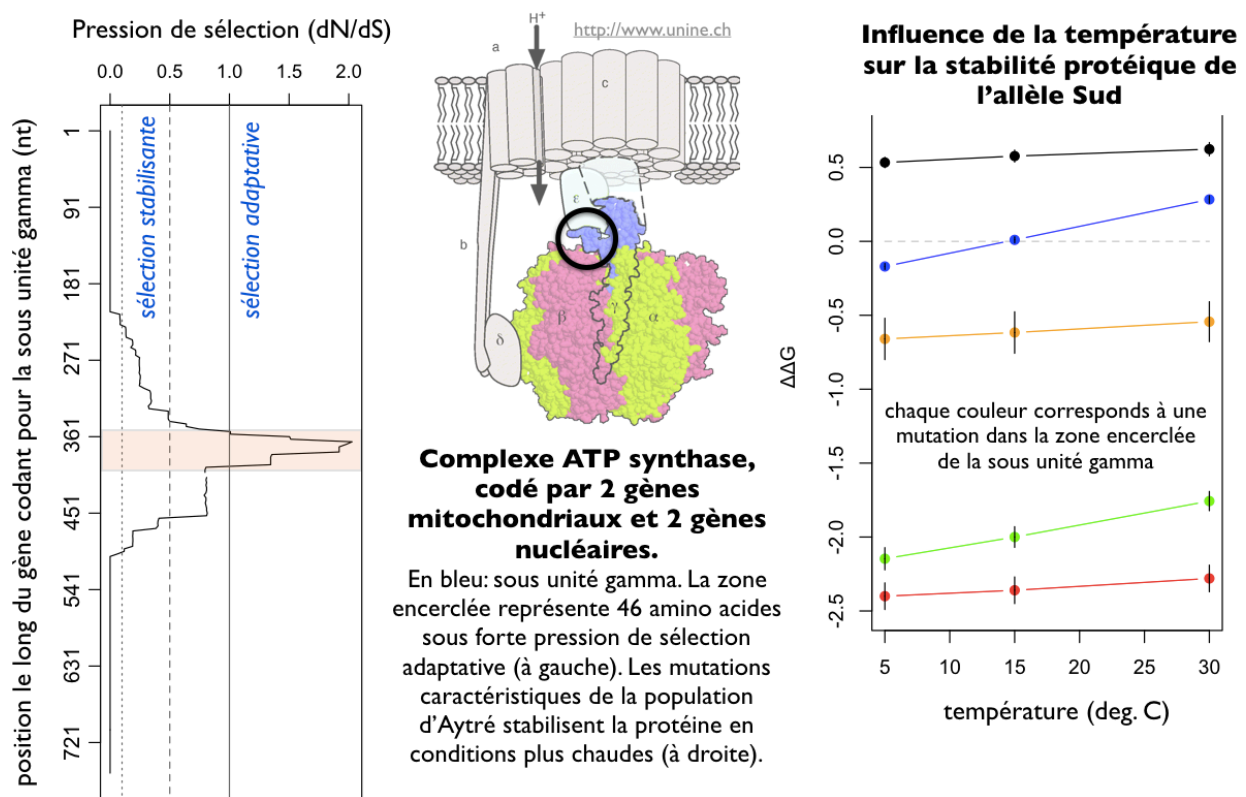
La détection de marqueurs SNPs dans le transcriptome de *L. balthica* de part et d'autre des zones hybrides Finistère et Baltique a permis, par F_{ST} scan, la détection de loci putativement sous sélection (Pante et al 2012a). Cependant, les méthodes de scan F_{ST} , qui sont de plus en plus utilisées pour détecter des loci putativement sous sélection et inférer une signature d'adaptation locale, peuvent souffrir d'un fort taux de faux positifs. Des loci détectés par ces méthodes peuvent donc être faussement liés à l'adaptation locale, quand d'autres scénarii expliquant leur forte structuration sont plus parsimonieux (e.g. structure hiérarchique des populations ou détection de barrières endogènes anciennes ; synthèse de Bierne et al 2011). Il est donc important de combiner les approches moléculaires pour trouver les cibles de la sélection.

Etant donné la divergence ancienne entre les lignées testées (rubra vs. balthica, et rubra nord vs. rubra sud ; voir plus haut) dans notre scan F_{ST} (Pante et al 2012a) et l'hypothèse biogéographique de contact secondaire (détaillé dans l'introduction), la détection de loci impliqués dans des barrières endogènes aux flux de gènes est probable. Parmi les gènes détectés comme fortement structurés par F_{ST} scan, plusieurs sont impliqués dans la synthèse de l'ATP, un processus qui nécessite la coopération de gènes mitochondriaux et nucléaires. Ce résultat suggère donc que des incompatibilités entre gènes mitochondriaux et nucléaires, typiques des zones hybrides (Maheshwari et Barbash 2011 ; Burton et Barreto 2012 ; Burton et al 2013), sont impliqués dans le maintien des barrières aux flux de gènes chez *L. balthica*.

J'ai exploré la structure du gène codant pour la sous-unité gamma du complexe ATP synthase FO-F1 (appelé *atp5c1* chez l'homme). La sous-unité gamma constitue le rotor du complexe ATP synthase, et doit interagir avec d'autres sous-unités pour que le complexe ATP synthase soit fonctionnel. Une analyse des pressions de sélection sur *atp5c1*, estimées grâce aux ratios des mutations non-synonymes et synonymes (ratio dN/dS), suggère que la séquence dans son ensemble est sous très forte pression de sélection négative (la grande majorité des mutations se trouve en dernière position des codons et n'influence pas la séquence de la protéine). En revanche, une analyse par fenêtre coulissante (voir ci dessous) suggère qu'une partie du gène, longue de 46 acides aminés, est sous forte pression de sélection positive. Quatre des 5 amino-acides mutés distinguent la lignée sud (population d'Aytré ; voir Figure 7) des populations nord (populations de la Baie de Somme et de la Baie de Gdansk). Différents exercices de modélisation suggèrent que ces mutations sont (1) non neutres mais stables d'un point

de vue évolutif (ces mutations sont retrouvées chez d'autres métazoaires), et (2) localisées dans une zone d'interaction inter-peptides (notamment avec la sous-unité epsilon, qui est en contact direct avec les sous-unités codées par des gènes mitochondriaux). Il est donc probable que le gène *atp5c1* soit impliqué dans des incompatibilités de type nucléaire-nucléaire et / ou mito-nucléaire et puisse donc limiter le flux de gènes entre lignées nord et sud Finistère. Les mutations diagnostiques de la lignée sud, cependant, semblent affecter la structure tertiaire de la protéine. Ainsi, la modélisation de la stabilité de la molécule (G) montre que les mutations sud ont un effet stabilisant sur la protéine en conditions plus chaudes (températures du sédiment dans la Baie de Gdansk et dans le sud du Golfe de Gascogne ; voir ci-dessous, Pante et al, en révision pour *Aquatic Living Resources*, actes du congrès international *PhysioMar17*; manuscrit en annexe 2).

Figure 7. Gènes de l'ATP synthase: incompatibilités génétiques entre lignées sud-nord Finistère, ou marqueurs de l'adaptation thermique?



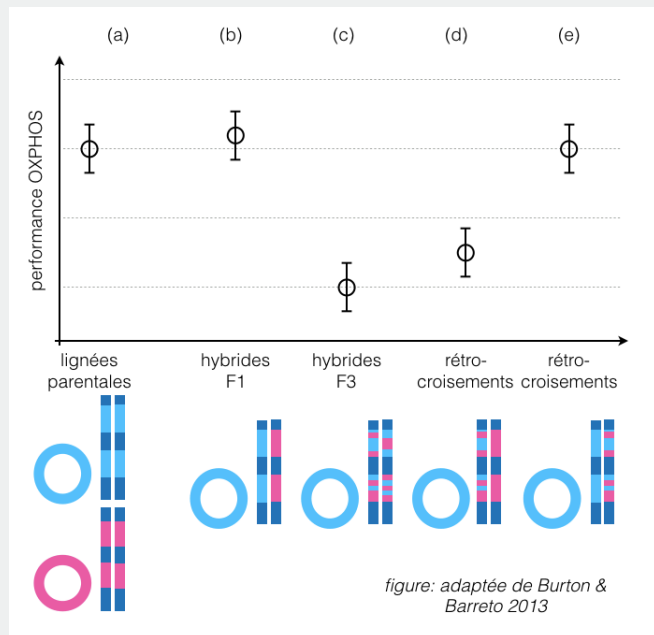
Le travail détaillé ci dessus sur le gène *atp5c1* illustre ce qui peut être fait à la suite d'un scan F_{ST} pour étudier l'implication des loci fortement différenciés dans l'établissement et le maintien des barrières aux flux de gènes. J'envisage de poursuivre la caractérisation du complexe ATP synthase de part et d'autre des zones hybrides ; notamment, d'obtenir des données de séquence pour les autres sous-unités nucléaires du complexe (voir Axe 2 ci dessous). L'assemblage du génome mitochondrial de *L. balthica* (Saunier et al 2014) constitue une première étape vers la détection d'éventuelles incompatibilités génétiques et pour évaluer les pressions de sélection qui agissent sur les gènes conduisant à la synthèse de l'ATP (e.g. Gagnaire et al 2012 ; détaillé dans l'Axe 2 ci dessous).

Dans le cadre du projet ANR blanc HySea (« Hybridization, a pivotal but neglected contributor to marine biodiversity dynamics », 2013-2015, porté par Frédérique Viard, Station Biologique de Roscoff) nous avons mis en place au laboratoire LIENSs les

Boite 2 - Suivi de la phénologie de la reproduction chez *Limecola balthica* et production d'hybrides en laboratoire

Afin de confronter nos données sur les pressions de sélection inférées sur les gènes de la chaîne OXPHOS de la mitochondrie, nous avons entrepris en 2013 de maintenir des individus en laboratoire afin de produire des hybrides de première et seconde génération, ainsi que des rétro-croisements (Saunier 2015). La production de ces individus permettra de valider expérimentalement l'implication d'incompatibilités génétiques mito-nucléaires dans le maintien des barrières aux flux de gènes chez *L. balthica*. Les attendus théoriques sur les performances physiologiques d'individus issus de populations parentales, d'hybrides et de rétro-croisements sont illustrés ci-dessous.

(a) Les mitogénomes (cercles) et chromosomes nucléaires (segments verticaux) sont ici représentés pour les lignées parentales (e.g. lignées sud et nord de la zone de contact Finistère / Cotentin). De nouvelles combinaisons génétiques entre gènes codant pour les sous-unités de la chaîne OXPHOS sont testés chez les hybrides **(bc)**. Une rupture de la co-adaptation mito-génomique mène à une diminution des performances mitochondriales **(c)** qui peuvent être rétablies lorsque les allèles nucléaires correspondant au contexte mito-génomique sont ré-introduits dans les rétro-croisements **(e)**. Un seul mitogénome est représenté ici, bien que les MNIs puissent se mettre en place entre génome nucléaire, et mitogénomes mâle et femelle.



Une première étape dans la mise en place de lignées maintenues en laboratoire est la description du cycle de reproduction en milieu naturel. En effet, bien que la phénologie de la reproduction ait été décrite en limite sud de l'aire de répartition chez *L. balthica* (site du Verdon, ~80 km au sud du laboratoire LIENSs; Bachelet 1980), il est possible que celle-ci ait été impactée par les réchauffement des eaux de surface du Golfe de Gascogne lors des 30 dernières années. En effet, le déclenchement de la ponte chez *L. balthica* est sensible aux changements climatiques (Phillipart et al 2003). Nous avons en effet observé que, sur le site d'Aytré, à quelques kilomètres au sud du laboratoire LIENSs, le déclenchement de la ponte a lieu 71 jours plus tôt que les observations faites par Bachelet en 1977 au Verdon (Saunier et al en préparation).

Ce travail de terrain nous a permis de pouvoir collecter des individus de *L. balthica* au maximum de maturité gonadique et au plus près du déclenchement de la ponte en milieu naturel. Il sert de fondation (1) aux expérimentations en laboratoire (production d'individus F1 lors de la thèse d'Alice Saunier), (2) aux études en populations naturelles (e.g. stage de M1 de Julie Brémaud sur la stabilité du mode de transmission de la mitochondrie).

infrastructures nécessaires pour croiser différentes lignées de *L. balthica* et tester les performances physiologiques (et notamment la production d'ATP) chez les lignées

parentales, les hybrides de première et seconde génération, et les rétrocroisements. Les tests de croisements se sont fait en collaboration avec Carl Van Colen (Université de Ghent) et différents membres du réseau HySea. Après plusieurs mois de mises au point, nous sommes parvenus à croiser les lignées nord- et sud-Finistère, et pourrions donc tester de façon expérimentale si des incompatibilités de type Dobzhanski-Muller sont responsables de la mal-adaptation des hybrides chez *M. balthica* (e.g. Burton et al 2013; voir Boite 2). Ce système expérimental nous permettra également de déterminer le rôle de l'environnement (et notamment de la température) dans l'expression des incompatibilités génétiques, un phénomène encore très peu étudié (Arnqvist et al 2010).

Egalement dans le cadre d'HySea, nous utilisons des banques BAC (Bacterial Artificial Chromosomes) afin de cartographier les zones chromosomiques putativement sous sélection (publication en préparation). Ces banques BACs ont été préparées par le Centre National de Ressources Génomiques Végétales (CNRGV, Toulouse) pour un individu d'Aytré (lignée *Limecola balthica rubra*) et un individu de Gdansk (lignée *Limecola balthica balthica*). J'ai utilisé les données du transcriptome de *L. balthica* (Pante et al 2012a) afin de produire des sondes pour capturer les fragments chromosomiques des banques BACs qui comportent des gènes putativement sous sélection (outliers F_{ST}), tels que les gènes impliqués dans la production et le transport de l'ATP (Pante et al 2012a). Une capture d'exons nous permettra de comparer de grands fragments chromosomiques à l'échelle des populations et des lignées, afin de déterminer (1) si les gènes impliqués dans la chaîne OXPHO de la mitochondrie sont véritablement la cible de la sélection, et (2) l'étendue chromosomique de la différenciation inter-populationnelle (i.e. architecture d'îlots de différenciation ; e.g. Wood et al 2008).

Axe 2 : Implication potentielle de la transmission uniparentale double de la mitochondrie dans le maintien de l'isolement reproducteur

Certaines espèces de bivalves présentent une exception remarquable à l'hérédité maternelle des mitochondries typique des métazoaires: le mode de transmission uniparental double ("doubly uniparental inheritance", ou DUI). Dans ce système, les femelles transmettent des mitochondries "femelles" (type F) qui persistent dans les ovocytes et dans les tissus somatiques des deux sexes, tandis que les mâles transmettent des mitochondries "mâles" (type M) qui persistent dans leur lignée germinale (Zouros, 2013; Figure 8). Ce système offre donc un énorme potentiel de développement pour les MNI dans les hybrides inter-populationnels, car le réseau d'interactions cyto-nucléaires est plus complexe (Saavedra et al 1996; Figure 9). Bien que des efforts importants aient été déployés ces dernières années pour comprendre le fonctionnement du DUI et son évolution (e.g. Breton et al 2007, 2014; Zouros 2013), peu d'informations sont disponibles sur la manière dont il pourrait contribuer à l'isolement reproducteur.

Nous avons montré la présence probable de DUI chez *L. balthica* (Pante et al 2016) en détectant des haplotypes mitochondriaux différents (26% de divergence au locus mitochondrial *rnL*) dans les tissus somatiques et gamétiques issus d'un même individu. Nous avons récemment confirmé la présence de DUI chez *L. balthica* en séquençant le mitogénome de type M au complet (manuscrit en préparation; voir section 3 ci dessous). Ce résultat est particulièrement intéressant, car en présence de DUI, des incompatibilités mito-génétiques peuvent être exprimées non seulement entre les gènes

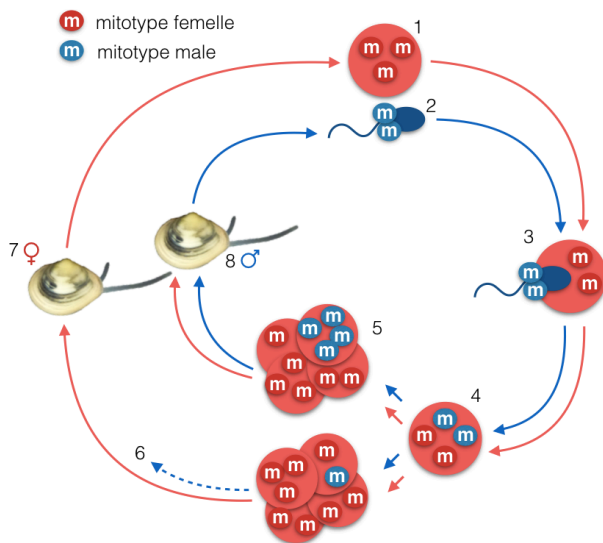
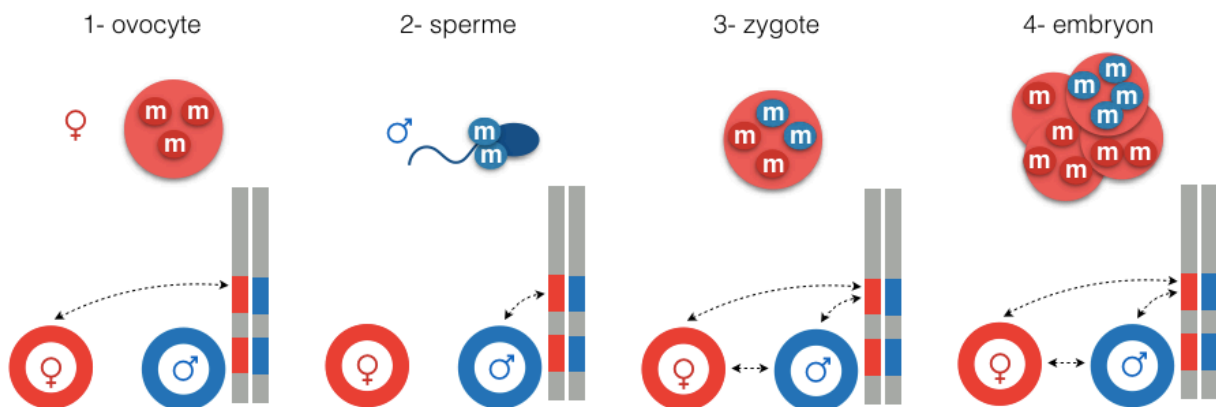


Figure 8. Modèle de la transmission uniparentale double de la mitochondrie chez les bivalves. L'ovocyte (1) et le spermatozoïde (2) portent un mitogénome (ou mitotype) unique, et sont donc homoplasmiques. Les mitochondries portant le mitotype mâle sont transmises à l'ovocyte au moment de la fécondation (3), le zygote est donc hétéroplasmique (4). Au cours des premiers stades de développement, les mitochondries portant le mitotype mâle se concentrent dans la lignée germinale des mâles (5), et se dispersent (jusqu'à devenir indétectables) chez les femelles (6). Au stade adulte, les femelles transmettent le mitotype F aux ovocytes, et les mâles le mitotype mâle aux spermatozoïdes (8) (figure adaptée de Breton et al 2014).

Figure 9. Relations épistatiques mito-nucléaires prédites dans le cas d'une transmission uniparentale double de la mitochondrie. Les flèches en pointillés représentent les relations épistatiques. Les différents stades de vie (1-4) correspondent aux stades décrits dans la Figure 8. Les flèches symbolisant les relations entre mitogénomes rappellent la recombinaison possible entre ces génomes.



mitochondriaux de type F et les gènes nucléaires (dans les tissus somatiques et les ovocytes chez les femelles), mais aussi entre les gènes mitochondriaux de type M et les gènes nucléaires dans le sperme des hybrides inter-populationnels. En théorie alors, l'hybridation pourrait potentiellement perturber toutes les fonctions clés du fonctionnement mitochondrial chez les espèces DUI, telles que la traduction, la transcription et la réplication de l'ADNmt par la production d'ATP (Burton et Barreto 2012). Ce mécanisme possible, bien que reconnu dans la littérature (Saavedra et al 1996), n'a pas, à ma connaissance, été explicitement testé. Cela pourrait toutefois avoir une incidence considérable sur le maintien de la divergence génétique entre les populations d'espèces bivalves hautement dispersives et fournir des informations clés sur l'établissement et le maintien de l'adaptation locale dans ce groupe taxonomique fortement diversifié.

Dans le modèle DUI, on espère observer des femelles homoplasmiques caractérisées par le génome mitochondrial de type F (le type M peut être présent mais très rare, Zouros 2013): des incompatibilités génétiques entre mitogénome femelle et génome

nucléaire peuvent être exprimées. Chez les mâles hétéroplasmiques, les mitochondries de type F sont localisées dans les tissus somatiques alors que les types M sont dans la lignée germinale (Figure 8). Comme les spermatozoïdes ne portent *a priori* que le type M, un différent jeu d'INM peut être exprimé. De plus, les embryons portent les deux types (même les embryons femelles, pendant une courte période de temps), menant à des interactions épistatiques encore plus complexes entre les génomes mitochondriaux et nucléaires. Il y a donc plusieurs étapes clés du cycle de vie des bivalves gonochoriques au cours desquelles le DUI peut mener aux INM, et le nombre de combinaisons MNI est plus élevé chez les espèces DUI que chez les espèces ayant une hérédité mitochondriale strictement maternelle ("strictly maternal inheritance" ou SMI). Dans la plupart des espèces DUI étudiées, le type M évolue plus rapidement que le type F (examiné dans Zouros 2013), fournissant un substrat plus riche pour les INM. La découverte du DUI chez *L. balthica* constitue donc une étape clé dans notre compréhension de la façon dont les obstacles au flux de gènes sont générés et maintenus chez cette espèce.

Pour répondre à ces questions, j'ai déposé en 2016 et 2017 un projet ANR JCJC, intitulé "DRIVE", visant à déterminer si le mode d'hérédité doublement uniparental de la mitochondrie peut jouer un rôle significatif dans le maintien des barrières au flux de gènes par le biais d'incompatibilités génétiques mito-nucléaires. Le projet DRIVE a été sélectionné pour financement en juillet 2018.

Dans le cadre de DRIVE, je propose (1) de séquencer l'ensemble du mitogénome mâle issus des différences lignées européennes, (2) de déterminer, par RNA-seq, si les isoformes nucléaires spécifiques du fond mitogénomique mâle sont exprimées dans les spermatozoïdes, et (3) d'estimer le degré de différenciation et le déséquilibre de liaison entre les gènes nucléaires et mitochondriaux (M et F) codant pour des complexes protéiques impliqués dans la production d'ATP (c'est-à-dire la chaîne de phosphorylation oxydative, ou OXPHOS).

Notre objectif de rechercher des isoformes nucléaires OXPHOS dans des spermatozoïdes purifiés est en soi nouveau et ambitieux, car aucune caractérisation du transcriptome des spermatozoïdes chez les espèces bivalves (et très peu chez les invertébrés) n'est disponible à ce jour.

(i) Mise au point de protocoles d'indication de ponte ; phénologie de *L. balthica*

L'étude du DUI repose sur la disponibilité d'individus sexuellement matures à partir desquels nous pouvons collecter des gamètes en quantité suffisante pour permettre des analyses génétiques. Nous avons étudié la phénologie de la reproduction chez *L. balthica* au voisinage du laboratoire (Saunier 2015; Saunier et al. en préparation), où nous collectons des individus sexuellement matures de la mi-mars au début mai. Le développement des gamètes et le déclenchement de la ponte en milieu naturel étant étroitement liés à la température de l'eau de mer, le développement gamétique est relativement prévisible. Néanmoins, comme la période de temps disponible pour collecter les échantillons et travailler sur la collecte des gamètes est étroite (les individus restent sexuellement matures et prêt à pondre pendant quelques semaines), nous avons travaillé ces cinq dernières années à améliorer nos protocoles pour maintenir les spécimens en laboratoire, contrôler le rythme de la maturation sexuelle, induire la ponte, et obtenir des individus hybrides (voir Boîte 2). Ainsi, en 2018, nous avons pu provoquer la ponte dans le laboratoire à fin juin, 3 mois après le pic de ponte observé sur le terrain.

(ii) Tests de construction de banques RNA-seq à partir d'échantillons gamétiques

Travailler sur des échantillons gamétiques, en particulier chez les bivalves de petite taille, peut être difficile en raison de la faible quantité de matériel biologique collecté chez des individus isolés. Au cours des deux dernières années, en préparation du projet ANR DRIVE, j'ai testé plusieurs méthodes d'obtention de sperme et d'épuration de l'ADN et de l'ARN (une bibliothèque de test RNA-seq du sperme a été séquencée avec succès). Bien que l'obtention de spermatozoïdes à partir de dissections nous permette d'obtenir suffisamment d'ADN et d'ARN pour préparer des banques génomiques, la contamination par les cellules somatiques persiste. Nous avons donc développé une méthode permettant d'obtenir des spermatozoïdes purs et de réduire significativement (ou d'éliminer) la contamination somatique en induisant la ponte et en concentrant l'éjaculat afin d'obtenir suffisamment d'ARN pour un séquençage de type RNA-seq.

Les spermatozoïdes ont longtemps été considérés comme des cellules hautement spécialisées dans lesquelles l'expression des gènes est extrêmement limitée voire inexistante. Ce point de vue, étayé par des données provenant d'espèces à fécondation interne, a été contesté par la transcriptomique (Miller 2014). Ceci reste à prouver chez les espèces à fertilisation externe comme les bivalves. Même si l'ARN du sperme est résiduel à la gamétogenèse, il nous renseignera sur l'interaction entre les gènes OXPHOS mitochondriaux de type M et les gènes OXPHOS nucléaires.

(iii) Séquençage du mitogénome mâle

L'obtention du mitogénome mâle complet est nécessaire à la caractérisation des interactions mito-nucléaires et leur implication potentielle dans les barrières aux flux de gène. Comme énoncé plus haut, ces interactions peuvent impacter les processus de production d'ATP (via la chaîne OXPHOS), de translation (via les 22 ARNt contenus dans le mitogénome; Saunier et al 2014), de transcription et de réplication (via notamment la région contrôle de la mitochondrie)(Burton et Barreto 2012).

Une première tentative de séquençage du génome mitochondrial mâle à partir d'une approche "shotgun" (séquençage non discriminant de toutes les molécules d'ADNc présentes dans un échantillon de gonade mâle issu de la population française d'Aytré) a été infructueuse, les lectures des mitogénomes mâles et femelles (provenants de contamination somatique) se mélangeant à l'assemblage. J'ai donc opté pour un séquençage 3ème génération (technologie "long read") en utilisant l'instrument Minion d'Oxford Nanopore. Cette technologie m'a permis d'assembler les deux mitotypes sans problème d'association chimérique des lectures (Capt, Breton et Pante; article en préparation). L'assemblage du mitogénome femelle, issue d'une forte contamination somatique inévitable lorsqu'on prélève les gamètes mâles par dissection et non par induction de ponte, nous a permis d'étalonner notre protocole d'analyse de données "long read", le mitogénome femelle de *L. balthica* étant déjà caractérisé (Saunier et al 2014).

Les premiers résultats de cette étude suggèrent :

- la présence probable d'un gène codant pour l'*atp8*, souvent décrit comme rare chez les bivalves, mais également détecté sur le mitogénome femelle.
- un mitogénome très long, de 25 kbp, dû à un gène *cox2* lui-même très long (4938 bp). L'extension *cox2*, semble être fonctionnelle puisque présente dans le transcriptome de *L. balthica* (Pante et al 2012a et données Illumina non publiées)

- une forte divergence des mitogénomes F et M issus d'un même individu (>30%).

(iv) Disruption du mode de transmission mitochondriale en zone d'hybridation

En plus du réseau complexe d'interactions épistatiques qui peuvent avoir lieu entre les génomes nucléaires et mitochondriaux chez les bivalves DUI, les mécanismes de l'hérédité mitochondriale peuvent être perturbés par une hybridation interspécifique. Chez les hybrides des moules du genre *Mytilus*, la perturbation DUI peut produire des femelles hétéroplasmiques et à des mâles homoplasmiques pour le type F. De telles perturbations ont été observées en populations naturelles (e.g. Rawson et al 1996, Smietanka et Burzynski 2017) ainsi que dans des croisements expérimentaux (Zouros 1994, Wood et al 2003). Par exemple, Rawson et al. (1996) ont détecté 4% de femelles hétéroplasmiques et 11% de mâles dépourvus du type M parmi les hybrides de *M. edulis* x *M. galloprovincialis*. Les répercussions fonctionnelles de la perturbation du mode de transmission de la mitochondrie ne sont cependant pas bien comprises. Nous savons cependant que chez *Mytilus* : (1) la répartition DUI affecte les sex-ratios (examinés dans Zouros 2013), (2) la mobilité des spermatozoïdes varie selon le contexte mitochondrial (Jha et al 2008) et les adultes peuvent montrer des schémas complexes d'hétérosis positif et négatif (par exemple Beaumont et al 2003, Shields et al 2008). Les auteurs ont postulé que les MNIs sont responsables de la perturbation du DUI chez les hybrides (par exemple, Rawson et al 1996). À ma connaissance, la perturbation DUI n'a pas été signalée dans les zones hybrides intra-spécifiques.

En mars-mai 2018, dans le cadre du stage de M1 de Julie Brémaud (Université de Bordeaux), nous avons cherché à échantillonner les populations de *L. balthica* dans la zone hybride intraspécifique française afin de rechercher des signes de perturbation DUI par amplification par PCR du gène *cox1* de type F et M des tissus gonadiques et somatiques des mâles et les femelles. Au total, huit populations ont été échantillonnées entre la Charente-Maritime et la Baie de Somme, aux extrémités et dans la zone d'hybridation française, avec 9 à 50 individus par sexe sur chaque site (total de 423 individus). Par approche PCR classique, nous n'avons détecté ni femelle hétéroplasmique, ni mâle homoplasmique, donc pas de disruption visible du mode de transmission de la mitochondrie dans la zone d'hybridation. Ce travail, qui prendra la forme d'un stage de M2 en janvier 2019, sera complété par une approche de PCR quantitative, plus sensible.

(v) Détection d'isoformes nucléaires spécifiques au mitogénome mâle et détection d'incompatibilités entre le mitogénome mâle et le génome nucléaire

Bien que l'instabilité du DUI chez les hybrides de *Mytilus* soit connue depuis la découverte du DUI, l'implication spécifique des INM dans la génération et le maintien des barrières au flux génétique chez les espèces DUI reste à tester explicitement (Smietanka et Burzynski 2017). La mise au point d'outils génomiques tels que le RNA-seq et l'enrichissement ciblé peuvent constituer des atouts importants pour analyser le répertoire des gènes mitochondriaux et nucléaires impliqués dans les fonctions mitochondriales et tester, dans les zones hybrides, les interactions entre DUI et MNI. Dans le cadre du projet DRIVE, nous testerons les hypothèses selon lesquelles (1) des isoformes OXPHOS nucléaires spécifiques aux mitotypes F et M sont exprimés dans les tissus somatiques et germinaux, respectivement, et (2) ces isoformes portent la signature moléculaire d'incompatibilités mito-nucléaires. Nous nous baserons sur un séquençage RNA-seq de gamètes purifiées (issues des tests d'induction de ponte) et de

tissus somatiques pour tester la première hypothèse. Nous comparons les données issus du transcriptome OXPPOS nucléaires aux données mitogénomiques M et F à une échelle populationnelle pour tester la seconde. Ce travail se déroulera entre 2019 et 2021 et fera l'objet d'un contrat postdoctoral.

Axe 3 : Diversité génétique et structure des populations de *Limecola balthica* avant et pendant son retrait des côtes espagnoles

La compréhension des répercussions évolutives de la contraction de l'aire de répartition de *Limecola balthica* n'est pas envisageable sans l'intégration des paramètres démographiques et génétiques historiques et contemporains. Ainsi, je propose, à moyen voire long terme, d'utiliser des échantillons collectés jusqu'en Galice, avant la disparition de *Limecola* sur ces côtes. Ces échantillons seront utilisés dans une analyse phylogéographique visant à déterminer (i) le nombre de stocks génétiques, (ii) la taille efficace de ces populations, et (iii) la diversité génétique, et cela avant la contraction de l'aire de répartition. L'estimation de la quantité de diversité génétique perdue depuis les années 1970 nous permettra de mieux appréhender les changements futurs au sein des populations de *Limecola balthica* au nord des Pertuis Charentais.

Je suis en contact avec plusieurs experts de *Limecola balthica* afin de mettre au point une banque de tissus. Plus de 280 échantillons de l'Atlantique NE et NW, ainsi que du Pacifique Nord, collectés entre 1880 et 1990, sont inventoriés dans les collections de plusieurs muséums européens et nord-américains. En outre, Guy Bachelet (CNRS, Station Marine d'Arcachon) nous a gracieusement cédé ses collections de tissus de *L. balthica*, collectés aux alentours de l'estuaire de la Gironde dans les années 1970 et 1980.

Deux approches seront privilégiées pour l'analyse de la structure génétique historique des populations. D'une part, j'utiliserai des gènes mitochondriaux dont le choix sera motivé par les résultats des investigations décrites dans les axes 1 et 2. Le séquençage de ces fragments de gènes nous permettra d'évaluer la diversité génétique et éventuellement de détecter des haplotypes absents chez les individus contemporains. Le génome mitochondrial est en général beaucoup plus variable que le génome nucléaire, ce qui rend cette molécule particulièrement intéressante en génétique des populations (e.g. Avise 2004). Également, plusieurs milliers de copies sont généralement présentes dans chaque cellule somatique, ce qui augmente la probabilité de pouvoir extraire et amplifier de l'ADN dégradé. De récents développements méthodologiques permettent le génotypage de spécimens anciens et/ou préservés dans de mauvaises conditions (e.g. Skage et al 2007). Une seconde approche consistera à génotyper un nombre restreint de SNPs nucléaires, choisis à partir des résultats des axes 1 et 2. Ainsi, nous pourrons utiliser des SNPs sous sélection pour évaluer les pressions sélectives passées.

Parce que travailler sur des spécimens muséologiques reste un exercice périlleux, du fait de l'âge des échantillons et de leur préservation parfois peu compatible avec un travail moléculaire (e.g. fixation des tissus au formol), j'ai démarré en 2013 une nouvelle collection d'individus de *L. balthica* des côtes françaises. En effet, les échantillons de la collection BioCoMBE (détails plus haut) ont été prélevés entre 1997 et 2007 (Becquet et al 2012, 2013). Cette série temporelle combinée (1997-2018) nous permet donc d'avoir des spécimens issus des mêmes sites, collectés à près de 20 ans d'écart, ou l'équivalent de 10 à 20 générations (le temps de génération de *L. balthica* étant compris entre 1 et 2 ans ; Budd et Rayment 2001).

Axe 4 : Prédiction de l'impact des changements climatiques sur les populations de *Limecola balthica*

Le changement de la limite sud de l'aire de répartition de *Limecola balthica* offre un cadre de travail dynamique qui préconise l'utilisation d'outils de prédiction des altérations démographiques et génétiques des populations. Ainsi, nous ferons, en aval de nos analyses en cours, un travail théorique de modélisation de la trajectoire des populations de *Limecola* face aux changements climatiques à venir. Ce travail sera basé sur deux approches: (i) la prédiction de trajectoire à partir de corrélation entre variations organismiques (changements génétiques et démographiques) et environnementales (changements de la température des eaux de surface), et (ii) l'évaluation de scénarios de changements démographiques et génétiques grâce à des programmes de simulation tels que KernelPop (Strand et Niehaus 2007).

La première approche utilise les données sur le passé et le présent pour décrire le futur des populations de *Limecola balthica* dans le Golfe de Gascogne. Les données génétiques (fréquences alléliques enregistrées à différentes échelles spatio-temporelles) issues du travail mené en amont (objectifs 1 et 2) seront comparées aux données océanographiques issues de différents modèles.

- Le logiciel POPS (Jay et al 2012) permettra d'estimer la covariance entre la structure spatiale des données génétiques (proportions d'admixture, pour chaque individu géo-référencé) et la température des eaux de surface. La matrice de covariance estimée par POPS sera ensuite utilisée pour prédire les changements de la structure spatiale de la zone hybride de *L. balthica*, grâce à des projections de température (jusqu'à 2100). Cette méthode nécessite des données génétiques contemporaines (nos données SNP), ainsi que des données de températures présentes et futures. Ces dernières sont disponibles via la NOAA (Reynolds et al 2007) et le projet Européen ENSEMBLES (Hewitt & Griggs 2004).
- L'outil de classification « Random Forest » permettra de tirer parti de notre série temporelle de tissus de *L. balthica* (1997-2015) et de construire un modèle de distribution des lignées de *L. balthica* grâce aux données de température. Cette méthode est bien établie et robuste (Cutler et al 2007). Elle a récemment été utilisée avec succès pour modéliser le devenir des zones hybrides entre *Mytilus edulis* et *M. galloprovincialis* dans le Golfe de Gascogne (Hilbish et al 2012), en utilisant des échantillons collectés entre 1989 et 2007 (Coustau et al 1991 ; Daguin et al 2001 ; Bierne et al 2003 ; Hilbish et al 2012), soit 9 à 18 générations (le temps de génération de *M. edulis* étant compris entre 1 et 2 ans ; Tyler-Waters 2008). Les paramètres de l'étude pilote de Hilbish et al (2012) sont donc très similaires à celle de mon projet.

Cependant, ainsi que tout travail basé sur des corrélations, cette première méthode ne permet pas de mettre en évidence des mécanismes particuliers. Pour cette raison, coupler cette approche avec des simulations est une démarche particulièrement intéressante, parce qu'elles permettent de tester la vraisemblance de différents scénarios, et de déterminer quels paramètres démographiques et génétiques influencent le plus la trajectoire globale des populations.

Voici quelques scénarios qui pourront être testés à l'aide de simulations:

1. Limite de répartition en mouvement, zone de tension stationnaire
2. Zone de tension en mouvement, limite de répartition stationnaire

3. Zone de tension et limite de répartition en mouvement

Ainsi que quelques paramètres dont l'importance relative pourra être estimée :

1. La taille efficace des populations limitrophes et centrales
2. Le nombre de migrants échangés par génération entre ces populations
3. La dérive génétique et la force de la sélection

Les études en cours sur la structure génétique des populations de *L. balthica* vont nous fournir les données brutes sur la diversité génétique des sous populations, la structuration génétique à échelle spatiale fine, et une estimation de paramètres démographiques comme la taille efficace des populations. Ces paramètres viendront nourrir nos modèles de prédiction.

5. Encadrement et enseignement

a) Encadrement de thèse

Je détaille ici ma participation aux directions de thèses. Une liste complète des étudiants que j'ai encadrés est fournie dans mon CV, en annexe 1.

Participation à la direction de thèse d'Alice Saunier

Période: 2012-2015
Affiliation: équipe AMARE, laboratoire LIENSs
Titre de la thèse: Bases génétiques de la différenciation adaptative en milieu anthropisé chez *Macoma balthica*, un bivalve marin à fort flux génique
Directrice: Pascale Garcia

Publication(s) issue(s) du travail de thèse (auxquelles j'ai participé):

Pante E, Poitrimol C, Saunier A, Becquet V, Garcia P (2017) Putative sex-linked heteroplasmy in the tellinid bivalve *Limecola balthica* (Linnaeus, 1758). *Journal of Molluscan Studies* 83(1).doi:/10.1093/mollus/eyw038

Saunier A, Garcia P, Becquet V, Marsaud N, Escudié F, Pante E (2014) Mitochondrial genomes of the Baltic clam *Macoma balthica* (Bivalvia: Tellinidae): setting the stage for studying mito-nuclear incompatibilities. *BMC Evolutionary Biology*. doi:10.1186/s12862-014-0259-z

Présentation(s) issue(s) du travail de thèse (auxquelles j'ai participé):

Evolution, Austin, Texas, USA (2016) Saunier A, Becquet V, Pante E, Garcia P. Gene expression patterns of oxidative phosphorylation chain across and within a marine hybrid zone

2ème Colloque d'Ecophysiologie Animale, La Rochelle, France (2015) Saunier A, Garcia P, Becquet V, Huet V, Manero F, Mabilieu G, Pante E, Murillo. Etude de la morphologie mitochondriale chez le bivalve marin *Macoma balthica* : comparaison d'individus purs et hybrides

2ème Colloque d'Ecophysiologie Animale, La Rochelle, France (2015) Saunier A, Becquet V, Pante E, Garcia P. Oxidative phosphorylation gene expression across and within a marine hybrid zone

1er Colloque d'Ecophysiologie Animale, Lyon, France (2013) Saunier A, Pante E, Marsaud N, Escudié F, Becquet V, Garcia P. Mitochondrial genome of the Baltic clam *Macoma balthica*: setting the stage for studying mito-nuclear incompatibilities

Contexte: Alice Saunier a intégré l'équipe AMARE du LIENSs en 2012, sous la direction de Pascale Garcia. Etant en post-doctorat sous la tutelle de Pascale Garcia depuis 2011, j'ai participé à l'écriture du sujet de thèse, ainsi qu'à

l'encadrement. J'ai notamment été sollicité pour mes compétences en bioinformatique et analyses de données mito-génomiques et transcriptomiques. La thèse d'Alice a donné lieu à deux publications de rang A, dont une pour laquelle elle est premier auteur. Une publication sur la phénologie de la reproduction chez *L. balthica* est en cours de préparation.

Résumé: Dans un contexte environnemental anthropisé, fragmenté et soumis à un changement climatique rapide, l'appréhension des processus d'adaptation locale des organismes marins par l'étude de zones de contact entre taxa proches constitue une approche privilégiée. Dans ces zones, des génotypes hybrides persistent malgré un état de mal-adaptation liée à des incompatibilités génétiques endogènes et/ou des barrières exogènes. L'histoire biogéographique complexe de la telline baltique *Macoma balthica* fait émerger quatre zones hybrides européennes, dont l'une, localisée autour de la Pointe Finistère (France), est le résultat d'un contact entre deux stocks génétiques ayant divergé en allopatrie. Ces divergences sont susceptibles de rompre la co-adaptation entre génomes nucléaire et mitochondrial en raison de l'émergence d'incompatibilités mitonucléaires (MNIs). Ainsi, les sous-unités protéiques des cinq complexes de la chaîne OXPHO sont codées à la fois par des gènes nucléaires et mitochondriaux, et une co-évolution inter-génomique étroite est requise pour maintenir la production énergétique cellulaire. De précédentes données de transcriptomique dévoilent de probables MNIs chez *M. balthica* au niveau des complexes respiratoires I et V. Afin d'apporter des éléments de compréhension aux mécanismes de maintien des zones hybrides dans un contexte de pression anthropique, le présent travail se propose de tester l'hypothèse de putatives MNIs dans cette zone de contact. Pour cela, (i) six mitogénomes correspondant à cinq lignées haplotypiques divergentes en Europe ont été séquencés et l'architecture génomique a été étudiée conjointement à une cartographie des mutations des 13 gènes mitochondriaux, (ii) le niveau de transcription de 5 gènes nucléaires et 8 gènes mitochondriaux (complexe I à V) des individus hybrides a été comparé à celui des lignées parentales après détermination du statut d'hybridation de chaque individu (six populations françaises). A défaut d'apporter des éléments de réponses concrets quant à l'existence de MNIs chez *M. balthica*, et ses répercussions évolutives en terme de dépression d'hybridation, ce travail constitue un tremplin vers une étude approfondie de la zone hybride française en développant de nouveaux outils moléculaires, et de solides techniques expérimentales pour la conduite de futurs croisements artificiels.

Participation à la direction de thèse de Lucas Torres (2015-2018)

Période:	2015-2018
Affiliations:	équipe AGRIPOP, laboratoire CEBC équipe AMARE, laboratoire LIENSs
Titre de la thèse:	Vers une approche phylogénétique de la conservation : application aux pétrels (oiseaux marins)
Directeurs:	Vincent Bretagnolle (CEBC) et Eric Pante (LIENSs)

Publication(s) issue(s) du travail de thèse (auxquelles j'ai participé):

Torres L, Welch AJ, Zanchetta C, Chesser RT, Manno M, Donnadiou C, Bretagnolle V, Pante E (2018) Evidence for a duplicated mitochondrial region in Audubon's shearwater based on MinION sequencing. *Mitochondrial DNA Part A* doi: 10.1080/24701394.2018.1484116

Présentation(s) issue(s) du travail de thèse (auxquelles j'ai participé):

International Seabird Group Conference, Liverpool (2018) Mitochondrial phylogeography in seabirds, dealing with duplication, heteroplasmy and pseudogenes in the Procellariiformes

Evolution, Montpellier (2018) Torres L, Pante E, Bretagnolle V. What drives genetic divergence in seabird populations? The example of the *Puffinus lherminieri* complex

Evolution, Montpellier (2018) Torres L, Welch A, Zanchetta C, Bretagnolle V, Pante E. Mitochondrial genome evolution in seabirds, the case of heteroplasmy and gene duplication in Procellariiformes

GenoToul, Castanet-Tolosan, Journée "Long-Reads" (2017) Torres L, Welch A, Zanchetta C, Bretagnolle V, Pante E. MinION sequencing provides new insight on the evolutionary history of seabird mitochondrial genomes

Journées de la Systématique Française, Banyul-sur-mer (2017) Torres L, Welch A, Zanchetta C, Bretagnolle V, Pante E. Histoire et impact d'une région mitochondriale dupliquée chez les Procellariiformes

Contexte: Vincent Bretagnolle m'a proposé de co-encadrer une thèse sur la phytogéographie et phylogénétique des oiseaux marins dès mon recrutement au CNRS. Notre objectif était de coupler ses connaissances sur le système à mes compétences en phylogénétique et phylogéographie pour approfondir les connaissances sur le processus de spéciation chez les oiseaux marins.

Dans le cadre d'une première étude sur le complexe *Puffinus lherminieri* (puffins d'Audubon), Lucas a entrepris de décrire, à l'aide de marqueurs mitochondriaux, la structure phylogéographique au sein du complexe. La thèse de Lucas a pris un tournant inattendu, bien qu'extrêmement intéressant, lorsqu'il a découvert la présence d'hétéroplasmie et de duplication de gènes mitochondriaux au sein de *P. lherminieri*, ainsi que la prévalence de pseudogènes nucléaires d'origine mitochondriale (numt). Lucas a décrit ces patrons dans un premier article publié dans *Mitochondrial DNA part A*. Deux autres articles sont en préparation pour décrire (1) la structure phylogéographique du complexe, et (2) l'incidence d'utilisation de données mitochondriales en phytogéographie lorsque celles ci sont brouillées par des phénomènes comme la duplication de gènes, l'hétéroplasmie et les numts.

Résumé: Près de 40% des espèces d'oiseaux marins (majoritairement des pétrels) sont mondialement menacés. Les enjeux de conservation de ces oiseaux doivent être clarifiés, au moyen d'une meilleure connaissance de leur systématique et histoire évolutive. Par ailleurs les processus de différenciation des populations et la phylogéographie de nombreuses espèces de pétrels restent mal connus. Ce sont des organismes hautement mobiles, caractéristique supposée les affranchir de barrières géographiques à

la dispersion. Cependant, en dépit de cette mobilité, on observe parfois au sein d'une même espèce une différenciation de populations importante. Plusieurs explications sont avancées, notamment le caractère hautement philopatrique de certaines espèces qui pourrait supprimer partiellement ou totalement l'effet de la dispersion. Toutefois il est nécessaire d'approfondir les connaissances sur les mécanismes à l'origine de la différenciation chez ces espèces. L'objectif général de la thèse consiste à étudier les caractéristiques phylogénétiques et phylogéographique des Procellariidae afin de mieux décrire leur distribution actuelle et les processus de différenciation ayant eu cours dans cette famille, ainsi que d'identifier les taxons à forts enjeux de conservation.

Participation à la direction de thèse de Léa Demay (2018-2021)

Période: 2018-2021

Affiliations: équipe AMARE, laboratoire LIENSs

Titre de la thèse: Phylogénétique et phytogéographie des pétrels

Directeurs: Vincent Bretagnolle (CEBC) et Eric Pante (LIENSs)

Contexte: La thèse de Léa Demay, qui doit débuter en 2018, s'inscrira dans la continuité de celle de Lucas Torres, et visera à approfondir nos connaissances sur les mécanismes qui génèrent et maintiennent les barrières aux flux de gènes entre populations de pétrels à fort pouvoir dispersif.

b) Enseignement

Dès le début de mes études de Master (2003-2005), j'ai été amené à enseigner à la station Grice Marine Lab du College of Charleston (Caroline du Sud, Etats-Unis). Durant cette période, j'ai enseigné les TP/TD de Biologie Générale pour non biologistes (1ère année du programme de Bachelor du College of Charleston) ainsi que l'Ecologie et la Zoologie des Invertébrés pour les spécialistes (3ème année du parcours Biologie). J'ai dispensé les mêmes enseignements (Biologie Générale et Ecologie) durant mes études de doctorat (Université de Louisiane, 2006-2011). J'ai donc une expérience cumulée de sept semestres sur ces matières, à laquelle viennent s'ajouter différents cours magistraux en Ecologie, Zoologie, Génétique et Ecophysiologie, aux Etats-Unis et en France (Université de La Rochelle). Ces interventions représentent au total plus de 660 heures de présentiel.

Mon enseignement des TP de Zoologie des Invertébrés au College of Charleston, pendant mon Master, m'a amené à approfondir mes connaissances sur les organismes des zones intertidales de l'Atlantique nord-ouest. Chaque semaine, une journée était dédiée à la collecte d'organismes locaux servant aux séances de TP (observations et dissections). Une fois mon Master obtenu (fin 2005), j'ai été recruté par le Grice Marine Lab pour enseigner les TP/TD d'Océanographie (physique, chimique et biologique) du programme de Master en Biologie Marine pendant un semestre, avec une liberté de développement des activités (j'ai notamment développé un TD sur l'utilisation du logiciel «Ocean Data View» pour la cartographie de données océanographiques). A cette expérience s'ajoutent des cours magistraux sur l'écologie et l'évolution des organismes

marins (e.g. théorie de la biogéographie insulaire, conservation des communautés de coraux récifaux et profonds).

6. Collaborations

Je collabore actuellement avec différentes équipes de recherche, dont:

- les membres de l'équipe AMARE, du laboratoire LIENSs (e.g. Marie Vagner et Thomas Lacoue Labarthe) sur l'apport de la transcriptomique dans l'étude des performances physiologique des animaux marins (modèles poisson et sèche)
- le Centre d'Etudes Biologiques de Chizé (Vincent Bretagnolle) et le GenoToul de Toulouse sur la biogéographie des oiseaux marins
- le Muséum national d'Histoire Naturelle (Sarah Samadi), l'université de Marseille (Didier Aurelle) et la Smithsonian Institution (Stephen Cairns, USA), sur la biogéographie et la biodiversité des octocoraux de grande profondeur
- la Station Biologique de Roscoff (Frédérique Viard), sur la phylogénétique des cionés
- l'université de Lyon (Cyril Dégletagne), l'université de Montréal (Sophie Breton, Canada), le GénoToul et le Centre National de Ressources Génomiques Végétales de Toulouse sur la génomique des bivalves
- l'université de Tasmanie (Candice Undiedt, Australia) et le Harvey Mudd College (Andrea Quattrini, USA) sur la phylogénétique des coraux
- l'université de Birmingham (Stacy Krueger-Hadfield, USA) et le College of Charleston (Erik Sotka, USA) sur la biogéographie d'espèces introduites (modèles huître et algue)

Ma collaboration avec l'équipe de Sarah Samadi est la plus ancienne, puisqu'elle remonte à mon travail de thèse dans l'équipe de Scott France. En 2015, le MNHN m'a attribué le titre de Correspondant afin d'officialiser mes missions auprès du muséum, dont la participation aux campagnes de collecte (6 campagnes en mer), le tri et l'identification des spécimens (plus de 2300 déterminations depuis 2006), le travail de dissémination auprès de la communauté scientifique (e.g. co-signature de 4 articles scientifiques) et du grand public (e.g. blog de l'IRD pour la dernière campagne en date: <http://www.nouvelle-caledonie.ird.fr/toute-l-actualite/carnets-de-bord/kanadeep-2017> ; articles de vulgarisation scientifique: voir CV).

7. Expertise

Je suis rapporteur pour 26 journaux internationaux (dont *Molecular Ecology*, *Marine Ecology Progress Series*, et le *Journal of Biogeography*), ainsi que pour différents organismes financeurs (Hawaii Coral Reef Initiative Research Program, Polish National Science Center, Commission Flotte Hauturière, National Geographics, Conseil de recherches en sciences naturelles et en génie du Canada). J'étais également éditeur chez *Zootaxa* (sur invitation) entre 2012 et 2015.

J'ai évalué 58 articles, 6 programmes de recherche, et édité 15 manuscrits depuis 2008. Mon activité de rapporteur est détaillé sur le site Publons: <https://publons.com/author/652624/eric-pante#profile>

8. Synthèse

Les différents projets de recherches sur lesquels j'ai travaillé sont très diversifiés, tant au niveau des questions, des échelles (temps et espace), que des modèles biologiques, mais tous se rejoignent dans une approche commune consistant à intégrer différents types de données, à conjuguer différents outils et disciplines, afin d'arriver à une synthèse scientifique. Ainsi, dans le cadre de mes travaux sur les coraux scléractiniaux, j'ai par exemple couplé des données d'écologie, de simulations numériques et modélisation (Pante et al 2006), de dendrochronologie (Pante et al 2008), ou encore de biomarqueurs de stress physiologique (Dustan et al 2008) pour adresser des questions de structuration des communautés, et de résilience des populations. Fort de cette expérience, j'ai intégré phylogénétique et mitogénomique, écologie moléculaire, morphométrie et biogéographie pour délimiter des espèces de coraux (Pante et France 2010; Pante et Watling 2012; Pante et al 2013) et travailler sur la notion d'endémisme en milieu marin (Thoma, Pante et al 2009; Pante et al 2015a). Cet intérêt pour le croisement des disciplines m'a amené à intégrer le LIENSs, unité de recherche inter-institut et inter-disciplinaire, à travailler sur l'apport de la taxinomie intégrative en écologie moléculaire (Pante et al 2015c), et m'a permis de développer mon projet de recherche actuel. Celui-ci a l'ambition de combiner la génétique en populations naturelles, à la physiologie expérimentale et aux simulations numériques afin de mieux comprendre la mise en place de l'isolement reproducteur en milieu marin. A travers mon activité d'encadrement, je m'efforce de transmettre ce goût pour la biologie intégrative aux étudiants.

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Annexes

Annexe 1: *Curriculum vitae*

Annexe 2: Articles représentatifs des travaux antérieurs (Figure 3 et partie 3c)

Annexe 3: Article en révision pour le journal *Aquatic Living Resources* (actes du congrès international PhysioMar17: <https://physiomar17.com>)

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<http://epante.wordpress.com>

Intérêts de recherche

- Écologie évolutive des invertébrés marins benthiques
- Dynamique spatio-temporelle des populations et des communautés
- Étude des processus de l'adaptation locale en milieu marin
- Utilisation des nouvelles technologies de séquençage en écologie évolutive
- Phylogénétique, systématique et taxonomie intégrative
- Inventaire de la biodiversité en milieu marin profond
- Mode de transmission de la mitochondrie

Cursus universitaire

- **2011–2014** Post-doctorat, Laboratoire LIENSs, Université de La Rochelle, France
- **2006–2011** Philosophical Doctorate, Biologie Évolutive, University of Louisiana at Lafayette, USA
- **2003–2005** Master of Science, Biologie Marine, College of Charleston, USA
- **2001–2002** Bachelor of Science, Biologie Marine, College of Charleston, USA
- **2000–2001** Euro-American Institute of Technology, Biologie Marine, Sophia Antipolis, France
- **1998–2000** D.E.U.G. de Biologie, Université Louis Pasteur, Strasbourg, France

Résumé de l'expérience professionnelle (détails dans les pages qui suivent)

- Co-signature de 33 articles, 20 en (co)1er/dernier auteur
- Google Scholar: 1034 citations, $h_{2018} = 15$; Scopus: 660 citations, $h_{2018} = 14$
- Co-signature de 3 articles de sensibilisation du grand public (2 en premier auteur)
- 30 présentations à des congrès (total de co-signatures : 63), et 18 séminaires depuis 2002
- Participation aux comités de lecture de 26 journaux scientifiques (58 évaluations depuis 2008)
- Éditeur chez *Zootaxa* (2012-15) et *Frontiers in Marine Systematics and Taxonomy*
- >650 heures de cours données en biologie générale, zoologie, écologie, évolution et océanographie
- Participation à 9 campagnes océanographiques depuis 2008 (Atlantique, Pacifique, Antarctique)
- Travail de terrain aux Îles Caymans, aux Bahamas, dans l'océan Atlantique nord-ouest, dans la mer Méditerranée, à Madagascar, en Nouvelle-Calédonie, en Papouasie Nouvelle-Guinée, et en Océan Austral (Passage de Drake), au Canada et aux U.S.A.

Articles publiés dans des revues internationales à comité de lecture

Les [étudiants](#) co-auteurs que j'ai (co-)encadré sont en bleu.

33. [Torres L](#), Welch AJ, Zanchetta C, Chesser RT, Manno M, Donnadiou C, Bretagnol V, **Pante E** (**accepted**) Evidence for a duplicated mitochondrial region in Audubons shearwater based on MinION sequencing. *Mitochondrial DNA Part A* doi:10.1080/24701394.2018.1484116
32. Viricel A, Becquet V, Dubillot E, **Pante E** (2018) De novo assembly and functional annotation of the transcriptome of *Mimachlamys varia*, a bioindicator marine bivalve. *Marine Genomics* doi:10.1016/j.margen.2018.04.002
31. **Pante E**, [Poitrimol C](#), [Saunier A](#), Becquet V, Garcia P (2017) Putative sex-linked heteroplasmy in the tellinid bivalve *Limecola balthica* (Linnaeus, 1758). *Journal of Molluscan Studies* 83(1). doi:/10.1093/mollus/eyw038
30. Breitwieser M, Viricel A, Graber M, Murillo L, Becquet V, Churlaud C, Fruitier-Arnaudin I, Huet V, Lacroix V, **Pante E**, Le Floch S, Thomas-Guyon H (2016) Short-term and long-term biological effects of chronic chemical contamination on natural populations of a marine bivalve. *PLoS ONE* PLoS ONE 11(3): e0150184 doi: 10.1371/journal.pone.0150184
29. Castelin M, Van Steenkiste N, Gilmore S, **Pante E**, Harbo R, Lowe G, Merilees B, Frey M, Therriault T, Abbott C (2016) Use of integrative taxonomy and DNA barcoding for characterization of ecological processes structuring marine benthic community assemblages in British Columbia. *Molecular Ecology Resources*
28. **Pante E** (2016) Nomenclatural note on the homonymy between the octocoral genus *Dendrobrachia* Brook 1889 and the entoproct phylum *Dendrobrachia* Xian-Guan, Bergström, Xio-Ya and Jie 2006. *Zootaxa* 4107(4):598-598. doi:/10.11646/zootaxa.4107.4.8
27. **Pante E**, France SC, Gey D, Cruaud C, Samadi S (2015a) An inter-ocean comparison of coral endemism on seamounts: the case of *Chrysogorgia*. *Journal of Biogeography* 42(10): 1907-1918. *IF* = 4.86. doi: 10.1111/jbi.12564
26. **Pante E**, Abdelkrim J, Viricel A, Gey D, France SC, Boisselier MC, Samadi S (2015b) Use of RAD sequencing for delimiting species. *Heredity* 114: 450-459. *IF* = 3.80. doi: 10.1038/hdy.2014.105
25. **Pante E***, Puillandre N*, Viricel A*, Arnaud-Haond S, Aurelle D, Castelin M, Chenuil A, Destombe C, Forcioli D, Valero M, Viard F, Samadi S (2015c) Species are hypotheses: avoid basing connectivity assessments on pillars of sand. *Molecular Ecology* 24(3): 525-544 (*contributed equally). *IF* = 5.84. doi: 10.1111/mec.13048
24. **Pante E***, Schoelinck C*, Puillandre N (2015d) From Integrative Taxonomy to Species Description: one Step Beyond. *Systematic Biology* 64(1): 152-160. *IF* = 11.53 (*contributed equally) doi: 10.1093/sysbio/syu083
23. [Sabatier E](#), **Pante E**, Dussud C, Van Canneyt O, Simon-Bouhet B, Viricel A (2015) Genetic monitoring of pilot whales, *Globicephala* spp. (Cetacea: Delphinidae), stranded on French coasts. *Mammalia* [ahead of print] doi: 10.1515/mammalia-2013-0155 *IF* = 0.82
22. Samadi S, Puillandre N, Boisselier MC, Corbari L, Maestrati P, Mana R, **Pante E**, Thubaut J, Zuccon D, Hourdez S (2015) Patchiness of deep-sea communities in Papua New Guinea and potential susceptibility to anthropogenic disturbances illustrated by seep organisms. *Marine Ecology* 36(S1): 109-132. *IF* = 1.84 doi: 10.1111/maec.12204

21. [Saunier A](#), Garcia P, Becquet V, Marsaud N, Escudié F, **Pante E** (2014) Mitochondrial genomes of the Baltic clam *Macoma balthica* (Bivalvia: Tellinidae): setting the stage for studying mito-nuclear incompatibilities. *BMC Evolutionary Biology*. *IF* = 3.41. doi:10.1186/s12862-014-0259-z
20. **Pante E**, Pascal PY, Becquet V, Viricel A, Simon-Bouhet B, Garcia P (2014) Evaluating the genetic effects of the invasive *Ocenebra inornata* on the native oyster drill *Ocenebra erinacea*. *Marine Ecology* *IF* = 1.84. doi: 10.1111/maec.12208
19. Viricel A, **Pante E**, Dabin W, Simon-Bouhet B (2014) Applicability of RAD-tag genotyping for interfamilial comparisons: empirical data from two cetaceans. *Molecular Ecology Resources* 14(3): 597-605. doi: 10.1111/1755-0998.12206 *IF* = 5.63
18. **Pante E***, Simon-Bouhet B* (2013) marmap: a package for importing, plotting and analyzing bathymetric and topographic data in R. *PLoS ONE* 8(9): e73051. doi: 10.1371/journal.pone.0073051 (* authors contributed equally) *IF* = 3.73
17. **Pante E**, [Heestand Saucier E](#), France SC (2013) Molecular and morphological data support reclassification of the octocoral genus *Isidoides*. *Invertebrate Systematics* 27: 365-378. doi: 10.1071/IS12053 *IF* = 1.59
16. Becquet V, Lasota R, **Pante E**, Sokolowski A, Garcia P (2013) Effects of fine-scale environmental heterogeneity on local genetic structure in *Macoma balthica* (L.) from the Gulf of Gdansk (southern Baltic Sea). *Hydrobiologia* 714(1): 61-70. doi:10.1007/s10750-013-1530-9 *IF* = 2.21
15. **Pante E***, Rohfritsch A*, Becquet V, Belkhir K, Bierne N, Garcia P (2012a) SNP detection from *De Novo* transcriptome sequencing in the bivalve *Macoma balthica*: marker development for evolutionary studies. *PLoS ONE* 7(12):e52302. doi:10.1371/journal.pone.0052302 (*authors contributed equally) *IF* = 3.73
14. Becquet V, Simon-Bouhet B, **Pante E**, Hummel H, Garcia P (2012) Glacial refugium versus range limit: conservation genetics of *Macoma balthica*, a key species in the Bay of Biscay (France). *Journal of Experimental Marine Biology and Ecology* 432-433: 73-82. doi:10.1016/j.jembe.2012.07.008 *IF* = 2.48
13. **Pante E**, Corbari L, Thubaut J, Chan T-Y, Mana R, Boisselier M-C, Bouchet P, Samadi S (2012b) Exploration of the deep-sea fauna of Papua New Guinea. *Oceanography* 25(3):214-225 *IF*₂₀₁₁ = 2.99. doi:10.5670/oceanog.2012.65
12. **Pante E**, France SC, Couloux A, Cruaud C, McFadden CS, Samadi S, Watling L (2012c) Deep-sea origin and in-situ diversification of chrysogorgiid octocorals. *PLoS ONE* 7(6):e38357. doi:10.1371/journal.pone.0038357 *IF* = 3.73
11. **Pante E**, Dustan P (2012) Getting to the point: accuracy of point count in monitoring ecosystem change. *Journal of Marine Biology* vol. 2012, Article ID 802875, 7 pages. doi:10.1155/2012/802875
10. **Pante E**, Wating L. (2012) *Chrysogorgia* from the New England and Corner Seamounts: Atlantic-Pacific connections. *Journal of the Marine Biological Association of the United Kingdom* 92(5):911-927. doi: 10.1017/S0025315411001354 *IF* = 1.13
9. Watling L, France SC, **Pante E**, Simpson A (2011) Biology of deep-water octocorals. *Advances in Marine Biology* 60: 41-123. doi: 10.1016/B978-0-12-385529-9.00002-0 *IF* = 5.00
8. McFadden CS, Benayahu Y, **Pante E**, Thoma JN, Nevarez A, France SC (2011) Limitations of mitochondrial gene barcoding in Octocorallia. *Molecular Ecology Resources* 11(1):19-31. doi: 10.1111/j.1755-0998.2010.02875.x *IF* = 5.63

7. **Pante E**, France SC (2010) *Pseudochrysogorgia bellona* n. gen., n. sp.: a new genus and species of chrysogorgiid octocoral (Coelenterata: Anthozoa) from the Coral Sea. *Zoosystema* 32(4):595-612. doi: 10.5252/z2010n4a4 IF = 0.68
6. Thoma JN*, **Pante E***, Brugler MR and France SC (2009) Deep-sea octocorals and antipatharians show no evidence of endemism on NW Atlantic seamounts. *Marine Ecology - Progress Series* 397:25-35. doi: 10.3354/meps08318 (*authors contributed equally) IF = 2.64
5. Dustan P, Fauth J, **Pante E**, Banks K, Vargas-Angel B, Downs, C (2008) Using cellular diagnostics to link land-based sources of pollution with coral reef degradation in South Florida. *Proceedings of the 11th International Coral Reef Symposium*, Session 16:495-499
4. Baker TG, **Pante E**, Roumillat WA, Lesveque EG and de Buron I (2008) *Metamicrocotyla macracantha*, a polyopisthocotylid gill parasite of the striped mullet, *Mugil cephalus*: population dynamics in South Carolina estuaries. *Parasitology Research* 102(5):1085-1088. doi: 10.1007/s00436-008-0920-9 IF = 2.33
3. **Pante E**, King A and Dustan P (2008) Short-term decline of a Bahamian patch reef community: Rainbow Gardens Reef (Exuma Cays, Bahamas), 1991-2004. *Hydrobiologia* 596(1):121-132. doi: 10.1007/s10750-007-9062-9 IF = 2.21
2. **Pante E**, Adjeroud M, Dustan P, Penin L and Schrimm M (2006) Spatial patterns of benthic invertebrate assemblages within atoll lagoons: importance of habitat heterogeneity and considerations for marine protected area design in French Polynesia. *Aquatic Living Resources* 19:207-217. doi: 10.1051/alr:200602 IF = 0.92
1. Baker TG, **Pante E** and de Buron I (2005) Co-occurrence of *Naobranchia lizae* (Copepoda) and *Metamicrocotyla macracantha* (Monogenea), gill parasites of the striped mullet, *Mugil cephalus*. *Parasitology Research* 97(6):515-520. doi: 10.1007/s00436-005-1485-5 IF = 2.33

Manuscrits soumis à des revues internationales à comité de lecture

- **Pante E**, Becquet V, Garcia P — Investigation of selection pressures on ATP synthase genes across a hybrid zone in the marine bivalve *Limecola balthica* (soumis, *Aquatic Living Resources*, Proceedings of Physiomar17)
- Aurelle D, **Pante**, Sartoretto — “A curious medley of characters:” phylogenetic placement of the enigmatic octocoral *Dendrobrachia* Brook 1889. (soumis, *Zootaxa*)
- Vagner M, **Pante E**, Viricel A, Lacoue-Labarthe T, Zambonino-Infante JL, Quazuguel P, Dubillot E, Huet V, Le Delliou H, Lefrancois C, and Imbert Auvray N — Ocean warming combined with lower omega-3 nutritional availability impairs the cardio-respiratory function of a marine fish. (soumis, *Journal of Experimental Biology*)

Articles pour le grand public

3. **Pante E** (2015) À la recherche des mécanismes de l'adaptation en milieu marin. *Microscop* 72, June 2015.
2. **Pante E**, France SC (2009) Randonnée au fond des océans : l'exploration des monts sous-marins. *Le Courrier de la Nature* 247: 34-41.
1. Viricel A and **Pante E** (2007) Utiliser la génétique pour mieux décrire, comprendre et protéger la nature. *Le Courrier de la Nature* 235: 22-27.

Abstracts & Proceedings

3. Castelin M, Van Steenkiste N, Gilmore S, **Pante E**, Harbo R, Lowe G, Merilees B, Frey M, Therriault T, Abbott C (**2015**) Use of integrative taxonomy and DNA barcoding for characterization of ecological processes structuring marine benthic community assemblages in British Columbia. *Genome* 58(5): 204. *IF* = 1.65 Abstracts from 6th Int. Barcode of Life Conf.
2. France SC, **Pante E**, Brugler MR and Van der Ham JL (**2010**) On the evolution of deep-sea octocorals and antipatharians: patterns revealed from molecular phylogenies. *Integrative and Comparative Biology* 50(S1):e56. doi: 10.1093/icb/icq106 *IF* = 2.97
1. Fauth JE, Dustan P, **Pante E**, Banks K, Vargas-Angel B and Downs CA (**2006**) Final Report: Southeast Florida Coral Biomarker Local Action Study. Florida Department of Environmental Protection, Southeast Florida Coral Reef Initiative, "Land Based Sources of Pollution" Project 5 www.dep.state.fl.us/coastal/programs/coral/reports/LBSP/Project5-Biomarker_Final_Report.pdf

Chapitres de livres, rapports, thèses, articles de magazines...

7. Brumbaugh & 23 autres auteurs (**2014**) Bahamas. pp. 118-122 in Status and Trends of Caribbean Coral Reefs: 1970-2012. Editors: Jackson J, Donovan M, Cramer K, Lam V. Global Coral Reef Monitoring Network, International Union for the Conservation of Nature, Gland, Switzerland.. 243 pages.
6. **Pante E**, Chan T-Y, Mana R, Bouchet B, Samadi S and the participants of the Tropical Deep-Sea Benthos cruises in Papua New Guinea (**2014**) Exploring deep mysteries of Papua New Guinea. *Deep-sea Life* 3:21.
5. **Pante E**, Simon-Bouhet B (**2014**) New open-source tools for deep-sea mapping. *Deep-sea Life* 3:39.
4. **Pante E**, Debruyne R, Gey D, Boisselier MC, Samadi S (**2013**) Focus 8-2. Du code-barre ADN vers les NGS : développement de marqueurs RAD-tag chez la gorgone *Chrysogorgia*. p 47 in Faure D, Joly D, Salameitou S (eds.) Génomique Environnementale: une prospective de l'Institut Ecologie et Environnement. CNRS, 96 pages.
3. **Pante E** (**2011**) Biogeography and evolution of chrysogorgiid corals. Thèse de doctorat, University of Louisiana at Lafayette, Lafayette LA (USA). 222 pages
2. **Pante E** (**2005**) Temporal Variation in a Bahamian Patch Reef Community: The Decline of Rainbow Gardens Reef. Thèse de Master, College of Charleston, Charleston SC (USA). 152 pages
1. **Pante E** (**2003**) Spatio-temporal patterns of coral recruitment around Moorea Island, French Polynesia. École Pratique des Hautes Études, Université de Perpignan (EPHE-UMR CNRS 8046 "Récifs Coralliens"). Rapport, 57 pages

Programmation

Je suis le co-auteur et mainteneur du paquet R appelé **marmap**. Voici les documents liés au développement de **marmap**. (également, voir l'article publié dans *PLoS ONE*, cité dans la section Publications).

- **Pante E**, Simon-Bouhet B (**2015**) marmap manual. Comprehensive R Archive Network, 45 pages. <http://cran.r-project.org/web/packages/marmap/marmap.pdf>
- **Pante E**, Simon-Bouhet B (**2015**) Making and using bathymetric maps in R with marmap. Tutorial. Comprehensive R Archive Network, 9 pages. <http://cran.r-project.org/web/packages/marmap/vignettes/marmap.pdf>

- **Pante E**, Simon-Bouhet B (2015) Data import and export strategies in marmap. Tutorial. Comprehensive R Archive Network, 9 pages.
<http://cran.r-project.org/web/packages/marmap/vignettes/marmap-ImportExport.pdf>
- **Pante E**, Simon-Bouhet B (2015) Analysing bathymetric data in R with marmap. Tutorial. Comprehensive R Archive Network, 20 pages.
<http://cran.r-project.org/web/packages/marmap/vignettes/marmap-DataAnalysis.pdf>

Financements

Génomique des populations du bivalve Tellinidae Limecola balthica

- 2018 ANR JCJC “DRIVE” Rôle de la transmission uniparentale double de la mitochondrie dans le maintien de l’isolement reproducteur. €233,686
- 2018 ACI mobilité, Université de La Rochelle (déplacement dans le laboratoire de Sophie Breton, Université de Montréal) €1,522
- 2017 ACI JCJC, Université de La Rochelle: Rôle de la transmission uniparentale double de la mitochondrie dans le maintien de l’isolement reproducteur. €5,536
- 2013 Bourse FEDER, 18 mois de salaire & €17,000 de fonctionnement, avec Pascale Garcia

Biogéographie et phylogénétique des octocoraux

- 2012 Bourse InEE CNRS, Génomique Environnementale, €12,000, avec M.C. Boisselier & S. Samadi
- 2011 Lerner Gray Fund for Marine Research (American Museum of Natural History), \$1,458
- 2011 Bourse de campagne de terrain, Société Française d’Écologie, €750
- 2010 Student Government Association (Université de Louisiane à Lafayette), \$640
- 2010 Stueben Fund (Université de Louisiane à Lafayette), \$250
- 2007 Lerner Gray Fund for Marine Research (American Museum of Natural History), \$2196
- 2006 Sigma Xi Grants-in-Aid of Research Program, \$400
- 2006-11 Graduate Student Organisation (Université de Louisiane à Lafayette), \$2800

Dynamique temporelle d’une communauté de coraux aux Bahamas

- 2004 Slocum Lunz Foundation Research Grant, \$500
- 2004 Perry Institute of Marine Science Development Grant, \$8135, avec Philip Dustan

Bourses et récompenses

- 2016 Prime d’encadrement doctoral et de recherche (PEDR)
- 2010 Bourse de voyage du journal *Ecography* pour la 5ème conférence de l’IBS
- 2007 Bourse de voyage pour l’École Thématique en Écologie Tropicale 2007 à Madagascar
- 2007 2ème place : meilleure présentation orale (8th Graduate Student Symposium, Lafayette)
- 2006-08 Bourse de thèse, Université de Louisiane à Lafayette
- 2005 “Outstanding Graduate Award,” College of Charleston

- 2005 1ère place : meilleure présentation orale (8th Marine Research Colloquium, Charleston)
- 2004-05 “Presidential Summer Research Award in Marine Biology,” College of Charleston
- 2002 “Award for Excellence in Marine Biology,” College of Charleston
- 2002 “Academic Scholarship for International Students,” College of Charleston
- 2001-02 Bourse d’étude de l’Euro-American Institute of Technology (Sophia-Antipolis)
- 1998-04 Bourse d’étude de la Carpreca

Congrès & Colloques

Les [étudiants](#) co-auteurs que j’ai (co-)encadré et qui ont présentés leurs travaux ont en bleu.

63. **International Seabird Group Conference**, Liverpool (2018) Welch A, Zanchetta C, Ribout C, Bretagnolle V, Pante E Mitochondrial phylogeography in seabirds: dealing with duplication, heteroplasmy and pseudogenes in the Procellariiformes (*virtual poster présenté par L. Torres*)
62. **Evolution**, Montpellier (2018) [Torres L](#), **Pante E**, Viricel A, Solics J, Ribout C, Zino F, Shirihai H, Bretagnolle V. What drives genetic divergence in seabird populations? The example of *Puffinus lherminieri* complex (*poster présenté par L. Torres*)
61. **Evolution**, Montpellier (2018) [Torres L](#), Welch A, Zanchetta C, Bretagnolle V, Pante E. Mitochondrial genome evolution in seabirds, the case of heteroplasmy and gene duplication in Procellariiformes
60. **GenoToul**, Castanet-Tolosan, Journée “Long-Reads” (2017) [Torres L](#), Welch A, Zanchetta C, Bretagnolle V, Pante E. MinION sequencing provides new insight on the evolutionary history of seabird mitochondrial genomes (*oral présenté par L. Torres*)
59. **Journées de la Systématique Française**, Banyul-sur-mer (2017) [Torres L](#), Welch A, Zanchetta C, Bretagnolle V, Pante E. Histoire et impact d’une région mitochondriale dupliquée chez les Procellariiformes (*oral présenté par L. Torres*)
58. **PhysioMar17**, Cambridge UK (2017) **Pante E**, Becquet V, Viricel A, Garcia P. The role of mito-nuclear genetic incompatibilities in the maintenance of gene flow barriers in the marine bivalve *Limecola balthica* (*oral présenté par A. Viricel*)
57. **PhysioMar17**, Cambridge UK (2017) **Pante E**, Becquet V, Viricel A, Garcia P. Is DUI involved in the maintenance of genetic barriers in the bivalve *Limecola balthica*? (*poster présenté par A. Viricel*)
56. **Symposium Exploration des faunes marines tropicales profondes : Tropical Deep-Sea Benthos fête ses 40 ans**, Paris, France (2016) **Pante E** Contributions of TDSB to the study of the Octocorallia (Cnidaria: Anthozoa). (talk)
55. **Colloque DiPEE** Tours-Poitiers-Chizé-La Rochelle, Tours, France (2016) Viricel A, **Pante E**. Doit-on continuer à utiliser le RADseq pour la phylogénomique? (talk)
54. **Colloque de Génomique Environnementale**, Paris, France (2016) Viricel A, **Pante E**. Should we pursue RAD sequencing for phylogenomics? A review of the literature. (*oral par A. Viricel*)
53. **IsoBay**, Bilbao, Espagne (2016) [Saunier A](#), Guesdon S, Becquet V, Garcia P, **Pante E**. Preliminary evidence for a rapid phenological shift at the southern range limit of a sentinel marine species of the Bay of Biscay. (*poster présenté par P. Garcia*)

52. **Evolution**, Austin, Texas (2016) [Saunier A](#), Becquet V, **Pante E**, Garcia P. Gene expression patterns of oxidative phosphorylation chain across and within a marine hybrid zone. (*Lightning talk par A. Saunier*)
51. **Evolution**, Austin, Texas (2016) Viricel A, **Pante E**. Choosing between reduced representation and targeted enrichment for phylogenomics. (poster)
50. **Colloque de Génomique Environnementale**, Montpellier, France (2015) **Pante E**, Becquet V, Beydon G, Saunier A, Bergès H, Garcia P. A BAC library approach for studying loci potentially involved in adaptation in *Macoma balthica*. (poster)
49. **Colloque de Génomique Environnementale**, Montpellier, France (2015) Viricel A, Becquet V, **Pante E**, Stenger PL, Thomas-Guyon H. Apport du barcoding moléculaire pour les études d'écotoxicologie marine. (*poster présenté par Amélia Viricel*)
48. **Colloque de Génomique Environnementale**, Montpellier, France (2015) Viricel A, **Pante E**, Caurant F, Méndez-Fernandez P, Peltier H, Prieto R, Llavona Vallina A, Ridoux V, Rogan E, Simon-Bouhet B. Revisiting common dolphin population structure in the Northeast Atlantic using a population genomics approach. (*oral présenté par Amélia Viricel*)
47. **2ème Colloque d'Écophysiologie Animale**, La Rochelle, France (2015) [Saunier A](#), Garcia P, Becquet V, Huet V, Manero F, Mabillean G, **Pante E**, Murillo L. Étude morphologique mitochondriale chez le bivalve marin *Macoma balthica* : comparaison d'individus purs et hybrides (*poster présenté par Alice Saunier*)
46. **2ème Colloque d'Écophysiologie Animale**, La Rochelle, France (2015) [Saunier A](#), Becquet V, **Pante E**, Garcia P. Oxidative phosphorylation gene expression across and within a marine hybrid zone (*oral présenté par Alice Saunier*)
45. **2ème Colloque d'Écophysiologie Animale**, La Rochelle, France (2015) Viricel A, Becquet V, Pante E, Stenger PL, Breitwieser M, Thomas-Guillon H. Apports du barcoding moléculaire pour les études d'écophysiotoxicologie. (*oral présenté par Amélia Viricel*)
44. **Colloque de Restitution des projets APEGE et BDV de l'InEE**, Paris, France (2015) Pante E, Abdelkrim J, Viricel A, Gey D, France SC, Boisselier MC, Samadi S. Du barcode vers les NTS: développement de marqueurs RAD chez la gorgonne *Chrysogorgia*. (présentation orale)
43. **14th International Deep-sea Biology Symposium**, Aveiro, Portugal (2015) Delavenne J, **Pante E**, Cairns S, Kitahara M, Samadi S, Habitat characterization on New Caledonian seamounts (*oral présenté par Juliette Delavenne*)
42. **International Barcode of Life Conference**, Guelph, Canada (2015) Castelin M, Van Steenkiste N, Gilmore SR, **Pante E**, Harbo R, Lowe G, Therriault T, Abbott CL. Use of integrative taxonomy and DNA barcoding for characterization of ecological processes structuring marine benthic community assemblages in British Columbia (*oral présenté par Magalie Castelin*)
41. **145th Annual meeting of the American Fisheries Society**, Portland, Oregon (2015) Castelin M, Van Steenkiste N, Gilmore SR, **Pante E**, Harbo R, Lowe G, Therriault T, Abbott CL. From high-throughput integrative taxonomy to community ecology: diversity and distribution of littoral shelled gastropods and brachyuran crabs in British Columbia, Canada. (*oral présenté par Magalie Castelin*)
40. **Colloque DiPEE Tours-Poitiers-Chizé-La Rochelle**, Poitiers, France (2014) **Pante E**. Évaluation des capacités d'adaptation des espèces marines dans un contexte de modifications climatiques. (présentation orale)

39. **1er Colloque d'Écophysiologie Animale**, Lyon, France (2013) [Saunier A](#), **Pante E**, Marsaud N, Escudié F, Becquet V, Garcia P. Mitochondrial genome of the Baltic clam *Macoma balthica*: setting the stage for studying mito-nuclear incompatibilities (*poster présenté par Alice Saunier*).
38. **Colloque de Génomique Environnementale**, Rennes, France (2013) Cetacean work: Viricel A, **Pante E**, Simon-Bouhet B; Coral work: **Pante E**, Debruyne R, Gey D, Boisselier MC, Samadi S; Insect work: Cruaud A, Gautier M, Galan M, Meusnier I, Saun A, Genson G, Artige E, Deuve T, Rasplus JY. Applicability of RAD-tag genotyping beyond the inter-specific level: empirical data from three model systems (*présentation orale avec Amélia Viricel et Astrid Cruaud*).
37. **Colloque de Génomique Environnementale**, Rennes, France (2013) **Pante E**, Becquet V, Rohfrisch A, and Garcia P. Potential role of mito-nuclear genetic incompatibilities in the maintenance of barriers to gene flow across secondary contact zones in the bivalve *Macoma balthica* (*poster*).
36. **Journée de retours d'expériences et d'échanges sur les développements technologiques**, GenoToul, Auzerville, France (2013) **Pante E**, Saunier A, Bouchez O, Becquet V, et Garcia P. Séquençage MiSeq de huit mitogénomes du bivalve *Macoma balthica* pour l'étude de la nature des barrières au flux de gènes en Europe (*présentation orale*).
35. **14th Congress of the European Society for Evolutionary Biology (ESEB)**, Lisbon, Portugal (2013) **Pante E**, Becquet V, Rohfrisch A, and Garcia P. Strong selection on mitochondria-targeting proteins across secondary contact zones in *Macoma balthica* (*poster*).
34. **14th Congress of the European Society for Evolutionary Biology (ESEB)**, Lisbon, Portugal (2013) [Saunier A](#), **Pante E**, Becquet V, and Garcia P. Complete mitochondrial genomes of the Baltic clam *Macoma balthica* (Bivalvia: Tellinidae): variable gene arrangements between and within lineages and genetic incompatibility (*poster présenté par A Saunier*).
33. **Software and Statistical Methods for Population Genetics Research Summer School**, Aussois, France (2013) **Pante E**, Saunier A, Becquet V, and Garcia P. Characterization of secondary contact zones and hybridization patterns in the tellinid bivalve *Macoma balthica* (*poster*).
32. **2nd European Conference on Speciation Research (Frontiers of Speciation Research programme)**, Montpellier, France (2013) **Pante E**, Saunier A, Becquet V, Rohfrisch A, and Garcia P. Characterization of secondary contact zones and hybridization patterns in the tellinid bivalve *Macoma balthica* (*poster*).
31. **3ème réunion du GDR MarCo**, Marseille, France (2013) **Pante E**, Simon-Bouhet B. marmap: a package for analyzing bathymetric and topographic data in R (*présentation orale*).
30. **2nde réunion du GDR MarCo**, Roscoff, France (2012) **Pante E**, Rohfritch A, Becquet V, Bierne N, Garcia P. SNP detection from de novo transcriptome sequencing in the bivalve *Macoma balthica*: marker development for evolutionary studies (*présentation orale*).
29. **97th Annual Meeting of the Ecological Society of America**, Portland, OR (2012) **Pante E**, Johnson D, Haynes K. Geographic variation in the effect of drought on southern pine beetle outbreaks (*poster présenté par Derek Johnson*).
28. **International Meeting of the Society for Molecular Biology and Evolution**, Dublin, Ireland (2012) **Pante E**, Rohfritch A, Becquet V, Bierne N, Garcia P. Extreme SNP density on the genome of *Macoma balthica*, a model system for the study of marine hybrid zone dynamics (*poster*).
27. **International Meeting of the Society for Molecular Biology and Evolution**, Dublin, Ireland (2012) Becquet V, Rohfritch A, **Pante E**, Garcia P. *De novo* analysis of the transcriptome of the marine bivalve *Macoma balthica* with the 454 sequencing technology. (*poster*)

26. **5th International Symposium on Deep-Sea Corals**, Amsterdam, The Netherlands (2012) **Pante E**, Heestand EN, France SC. Is *Isidoides* a bamboo coral? Evidence from DNA phylogenies, mitochondrial gene order and morphology (*poster présenté par Scott France*)
25. **5th International Symposium on Deep-Sea Corals**, Amsterdam, The Netherlands (2012) **Pante E**, France SC, Samadi S. A global approach to the study of endemism on seamounts: the case of *Chrysogorgia* (*poster présenté par Scott France*)
24. **5th International Biogeography Conference**, Heraklion, Crete (2011) **Pante E**, France S, Samadi S. Endemism of deep-sea corals on NW Atlantic and SW Pacific seamounts (poster)
23. **11th Graduate Student Symposium** Lafayette, LA (2010) **Pante E**. Exploration of the deep-sea fauna of Papua New Guinea (présentation orale)
22. **12th International Deep-sea Biology Symposium**, Reykjavík, Iceland (2010) **Pante E**, France S, Watling L. Insights into the evolution and biogeography of chrysogorgiid corals (présentation orale)
21. **12th International Deep-sea Biology Symposium**, Reykjavík, Iceland (2010) **Pante E**, France S, Watling L, Samadi S. Biogeography of the pan-distributed deep-sea coral genus *Chrysogorgia* (poster)
20. **Workshop CEAMARC**, Hobart, Tasmania (2010) Collin A, Dawson M, McFadden C, France S, **Pante E**. Progress with the sorting and analyses of the benthos and the dermesal fish samples collected during CEAMARC on board the Aurora Australis : CEAMARC - Cnidaria (*présentation orale de Nadia Améziane*)
19. **SICB Annual Meeting**, Seattle, WA (2010) France SC, **Pante E**, Brugler MR, van der Ham J. On the evolution of deep-sea octocorals and antipatharians: Patterns revealed from molecular phylogenies (*présentation orale de Scott France*)
18. **10th Graduate Student Symposium** Lafayette, LA (2009) **Pante E**. Insights into the evolution and biogeography of chrysogorgiid corals (présentation orale)
17. **4th International Symposium on Deep-Sea Corals**, Wellington, New Zealand (2008) **Pante E**, France SC. The first molecular phylogenetic reconstruction of the Chrysogorgiidae (Anthozoa: Octocorallia) questions its monophyly (*poster présenté par Scott France*)
16. **4th International Symposium on Deep-Sea Corals**, Wellington, New Zealand (2008) France SC, **Pante E**. On the origin of deep-sea octocorals: patterns revealed from molecular phylogenies (*présentation orale de Scott France*)
15. **4th International Symposium on Deep-Sea Corals**, Wellington, New Zealand (2008) Thoma J, **Pante E**, Brugler MR, France SC. Deep-sea corals show no evidence of endemism on northwestern Atlantic seamounts (*présentation orale de Scott France*)
14. **4th International Symposium on Deep-Sea Corals**, Wellington, New Zealand (2008) Watling L, France SC, **Pante E**, Thoma J, Simpson A. Atlantic-Pacific relationships of deep-sea octocorals (*présentation orale de Les Watling*)
13. **11th International Coral Reef Symposium**, Fort Lauderdale, FL (2008) **Pante E**, Dustan P. Getting to the point: accuracy assessment of point counting to monitor degrading reefs. Poster presentation (*présentation de Phillip Dustan - poster différent de celui présenté au 4ème SEEC*)
12. **11th International Coral Reef Symposium**, Fort Lauderdale, FL (2008) Dustan P, Fauth J, Banks K, **Pante E**, Vargas-Angel B, Downs, C. Using cellular diagnostics to link land-based sources of pollution with coral reef degradation in South Florida. Oral presentation (*présentation orale de Phillip Dustan*)

11. **Ocean Sciences Meeting**, Orlando, FL (2008) **Pante E**, France SC. Phylogeography of deep-sea chrysogorgiid corals (Cnidaria: Octocorallia) from the New England and Corner Seamounts (western North Atlantic Ocean) (poster)
10. **8th Graduate Student Symposium** Lafayette, LA (2007) **Pante E** Advances in coral reef monitoring: from coral identification to pattern recognition (présentation orale)
9. **8th Graduate Student Symposium** Lafayette, LA (2007) **Pante E**, Viricel A. R: an open-source, multi-purpose tool for biologists (poster)
8. **4th Southeastern Ecology and Evolution Conference**. Orlando, FL (2007) **Pante E** Getting to the point: accuracy of ecological monitoring using “point count” in the context of ecosystem degradation (poster)
7. **2nd Southeastern Ecology and Evolution Conference**. Athens, GA (2005) **Pante E**, Dustan P. Temporal changes in the stony coral community of Rainbow Gardens Reef (Iguana Cay, Bahamas), 1991-2004 (présentation orale)
6. **8th Annual Marine Research Colloquium**. Charleston, SC (2005) **Pante E**, Dustan P. On the use of texture analysis as a tool to automate calculation of coral cover from video transects (présentation orale)
5. **1st Southeastern Ecology and Evolution Conference**. Atlanta, GA (2004) **Pante E**, Adjerdoud M. Spatial structure of coral assemblages within atoll lagoons of French Polynesia (poster)
4. **33rd Benthic Ecology Meeting**. Mobile, AL (2004) **Pante E**, Adjerdoud M. Spatial patterns of coral communities within atoll lagoons in the Tuamotu Archipelago (poster)
3. **American Society of Parasitologists**. Philadelphia, PA (2004) Baker TG, **Pante E**, Roumillat WA, Lesveque E, and de Buron I. Co-occurrence of the copepod *Naobranchia lizae*, and the monogenean *Metamicrocotyla macracantha* on the striped mullet *Mugil cephalus*. (*présentation orale de Tiffany Baker*)
2. **Southeastern Society of Parasitologists**. Mobile, AL (2003) France SC, Viricel A, **Pante E**, de Buron I. Sequencing of the mitochondrial COI gene in several species of acanthocephalans and an application to the teaching laboratory. (*présentation orale d’Isaure de Buron*)
1. **10th International Congress of Parasitology**. Vancouver, BC (2002) France SC, Viricel A, **Pante E**, de Buron I. DNA sequencing of acanthocephalans mitochondrial COI gene. (*présentation orale d’Isaure de Buron*)

Séminaires

18. 2016 Atelier bioinformatique d’utilisation de données de type RAD, Muséum national d’Histoire naturelle (MNHN), Paris, France
17. 2015 Intégrer taxonomie, phylogénétique et génétique des populations pour mieux comprendre la structure de la biodiversité. Séminaire de la Fédération de recherche en environnement et développement durable (FREDD), Université de La Rochelle (France)
16. 2014 Next-generation transcriptome and mitogenome sequencing for characterizing barriers to gene flow in the bivalve *Macoma balthica*. Next-generation technologies workshop, Muséum national d’Histoire naturelle (MNHN), Paris, France
15. 2014 RAD-tag sequencing for evolutionary studies. Bioinformatics Workshop, Alliance nationale pour les Sciences de la Vie et de la Santé (AVIESAN), Roscoff, France.

14. 2014 Randonnée au fond des océans: diversité et origines d'une faune unique. École de la Mer, Médiathèque Michel Crépeau, La Rochelle (France)
13. 2013 Bases génétiques du potentiel adaptatif du mollusque marin *Macoma balthica*, espèce clé des côtes atlantiques. Séminaire de la Fédération de recherche en environnement et développement durable (FREDD), Université de La Rochelle (France)
12. 2013 Genetic basis of the adaptive potential of the marine bivalve *Macoma balthica*, a key species of the French Atlantic coast. Séminaire à la Station d'Ecologie Expérimentale du CNRS à Moulis (France)
11. 2013 marmap: a package for analyzing bathymetric and topographic data in R. Séminaire de la Fédération de recherche en environnement et développement durable (FREDD), Université de La Rochelle (France)
10. 2013-18 What can we expect from Next Generation Sequencing methods for molecular taxonomy and DNA-barcoding projects? Pante E, Debruyne R, Samadi S. DNA-Barcoding course at the Muséum national d'Histoire naturelle (MNHN), Paris (France)
9. 2012 Randonnée au fond des océans. Muséum d'Histoire naturelle, La Rochelle (France)
8. 2011 Évolution et biogéographie des coraux de grandes profondeurs. Séminaire de la Fédération de recherche en environnement et développement durable (FREDD), Université de La Rochelle (France)
7. 2011 Le métier de chercheur en biologie de l'évolution. Lycée Scheurer-Kestner, Thann (France)
6. 2010 Randonnée au fond des océans: exploration of the deep oceans. Département des Langues Modernes, Université de Louisiane à Lafayette (USA)
5. 2010 Biogeography of the pan-distributed deep-sea coral genus *Chrysogorgia*. Département de Biologie, Université de Louisiane à Lafayette (USA)
4. 2009 Évolution et biogéographie des octocoraux d'eaux profondes. Muséum national d'Histoire naturelle (MNHN), Paris (France)
3. 2008 Diversité génétique des octocoraux des monts sous-marins de l'Atlantique nord-ouest. Institut de Recherche pour le Développement (IRD), Nouméa (Nouvelle-Calédonie)
2. 2008 From corals to lemurs : discovering Malagasy natural history. Département de Biologie, Université de Louisiane à Lafayette (USA)
1. 2007 Temporal Variation in a Bahamian Patch Reef Community: The Decline of Rainbow Gardens Reef. Département de Biologie, Université de Louisiane à Lafayette (USA)

Enseignement (plus de 660 heures de cours administrées depuis 2004)

En qualité de moniteur

- 2008 General Ecology (spécialité Sciences - BS), 1 semestre, ULL
- 2007 Introduction to Biology (cours pour les non-spécialistes - BS), 1 semestre, ULL
- 2006 Oceanography (cours de Master en Biologie Marine), 1 semestre, CofC
- 2005 Invertebrate Zoology (spécialité Sciences - BS), 1 semestre, CofC
- 2004 General Ecology (spécialité Sciences - BS) 1 semestre, CofC

- 2003-04 Introduction to Biology (cours pour les non-spécialistes - BS), 3 semestres, CofC

En qualité d'intervenant

- 2018 TD génétique des populations (L3), ULR
- 2014 *Mutations* (Génétique Fondamentale), ULR
- 2013-17 *Étude du transcriptome* (Ecophysiologie), ULR
- 2010 *Alternatives to “canned” software for phylogenetics* (Systematic Methods - PhD), ULL
- 2010 *Models of Evolution* (Systematic Methods - PhD), ULL
- 2010 *Demography* (Ecology - BS), ULL
- 2009 *Coral Conservation Biology* (Master 2 UNESCO), MNHN, Paris, France
- 2009 *Phylum Platyhelminthes* (Invertebrate Zoology - BS), ULL
- 2009 *Evolutionary Trees* (Genetics and Evolution - BS), ULL
- 2005 *The Degradation of Caribbean Coral Reefs* (Invertebrate Zoology - BS), CofC
- 2004-05 *The Theory of Island Biogeography* (General Ecology - BS), CofC

Co-direction de thèse avec V. Bretagnolle, CEBC

- 2018-21 Léa Demay, doctorat, Université de La Rochelle (*biologie moléculaire*)
- 2015-18 Lucas Torres, doctorat, Université de La Rochelle (*biologie moléculaire*)

Participation à la formation d'étudiants en doctorat

- 2012-15 Alice Saunier, doctorat, Université de La Rochelle (*biologie moléculaire*)
- 2016 Marine Malfant, doctorat UPMC (Roscoff, dir. F. Viard), séjour d'1.5 mois, *phylogénomique*
- 2010 Esprit Heestand, doctorat, ULL (*systématique, méthodes en biologie moléculaire*)
- 2008 Anne Simpson, doctorat, University of Maine (*méthodes en biologie moléculaire*)

Participation à la formation d'étudiants en BTS, Bachelor, Licence et Maîtrise

- 2018 Julie Brémaud, Master 1, Université de Bordeaux (*biologie moléculaire*)
- 2018 Isobel Cook, Licence 3, Université de La Rochelle (*morphométrie, biologie moléculaire*)
- 2018 Justine Pageault, BTS Lycée Valin, La Rochelle (*biologie moléculaire*)
- 2016 Camille Poitrimol, Licence 3, Université de La Rochelle (*biologie moléculaire*)
- 2014 Benjamin Vittrant, Master 1, Muséum national d'Histoire naturelle (*taxonomie*)
- 2013 Etienne Sabatier, Master 1, Université de Bretagne Occidentale (*biologie moléculaire*)
- 2011 Hannah Knott, Bachelor, ULL (*systématique, méthodes en biologie moléculaire*)
- 2005 Juliana Miller, Master, CofC (*méthodes en biologie expérimentale*)

ULR : Université de La Rochelle (France); ULL : Université de Louisiane à Lafayette (USA); CofC : College of Charleston (USA); BS : Cours de 1er cycle (Bachelor); PhD : Cours de 3ème cycle (doctorat)

Expérience de terrain : 160 jours passés en mer

- 2017 **Campagne “KanaDeep.”** 15 jours sur le navire *N/O Alis*. Chef de mission : Stéphane Hourdez (CNRS). Exploration des pentes océaniques et monts sous-marins de la Nouvelle-Calédonie
- 2017 **Campagne “BioMaGlo.”** 21 jours sur le navire *N/O Alis*. Chef de mission : Laure Corbari (MNHN). Exploration des pentes océaniques et monts sous-marins autour de Mayotte, Comores et Glorieuses
- 2012 **Campagne “Madang.”** 23 jours sur le navire *N/O Alis*. Chef de mission : Sarah Samadi (MNHN). Exploration des pentes océaniques et monts sous-marins de la Papouasie Nouvelle-Guinée
- 2011 **Campagne “ExBoDi.”** 17 jours sur le navire *N/O Alis*. Chef de mission : Sarah Samadi (MNHN). Exploration des pentes océaniques et monts sous-marins de la Nouvelle-Calédonie
- 2011 **Campagne “Historic perspectives on climate and biogeography from deep-sea corals in the Drake Passage.”** 34 jours sur le navire *R/V Nathaniel B Palmer*. Chefs de mission : Rhian Waller (Univ. Maine), Laura Robinson (WHOI) and Kathryn Scanlon (USGS). Exploration des récifs coralliens profonds du Passage de Drake (Océan Austral)
- 2010 **Campagne “BioPapua.”** 15 jours sur le navire *N/O Alis*. Chefs de mission : Laure Corbari (MNHN) et Sarah Samadi (IRD/MNHN). Exploration des pentes océaniques et monts sous-marins de la Papouasie Nouvelle-Guinée
- 2009 **Campagne “Bahamas Deep Corals.”** 18 jours sur le navire *R/V F.G. Walton Smith*. Chef de mission : Scott France (Univ. Louisiane à Lafayette). Exploration de l’escarpement des Bahamas
- 2008 **Campagne “Terrasses.”** 17 jours sur le navire *N/O Alis*. Chef de mission : Sarah Samadi (IRD/MNHN). Exploration des pentes océaniques et monts sous-marins de la Nouvelle-Calédonie
- 2008 **Campagne “Evolutionary processes in deep-sea molluscs.”** 17 jours sur le navire *R/V Endeavor*. Chef de mission : Ron Etter (Univ. Massachusetts Boston). Échantillonnage de mollusques profonds le long du “Gay Head–Bermuda transect” (pente continentale, Atlantique nord-ouest)
- 2007 **École Thématique en Écologie Tropicale:** Biodiversité Tropicale et Insulaire. Morondava, Madagascar, 2 semaines. Détails : <http://millsonia.free.fr/ETET2007.html>
- 2004 **Travail de Terrain dans le cadre du Master of Science :** Caribbean Marine Research Center (Perry Institute of Marine Science, Lee Stocking Island, Bahamas), 3 semaines. Écologie des communautés benthiques de Rainbow Gardens Reef
- 2002 **Stage de recherche** (“Structure and Diversity of Coral Reefs”) au Central Caribbean Marine Institute, en partenariat avec Rutgers University, à Petit Cayman, 3 semaines

Autres expériences professionnelles

- 2012-13 **Environmental Consultant** pour la société australienne Geo Oceans : “ecological monitoring of marine environments using remotely-operated vehicles”
- 2011 **Workshop on Comparative Genomics**, Colorado State University. 96 heures de formation sur les nouvelles technologies de séquençage de l’ADN et analyse des données
- 2010-11 **Assistant de recherche** de Derek Johnson (Univ. Louisiane à Lafayette) : “Role of drought in southern pine beetle outbreaks.” Analyse de données et programmation dans le logiciel R

- 2006-10 **Systématique et taxinomie**. Visites annuelles au Muséum National d'Histoire Naturelle ; identification et barcoding des cnidaires des campagnes IRD/MNHN "Tropical Deep-sea Benthos" (anciennement MUSORSTOM)
- 2009 **Assistant de recherche** de Scott France (Univ. Louisiane à Lafayette) : mise à l'essai de marqueurs microsatellites pour l'octocorail *Primnoa pacifica* des Îles Aléoutiennes, Alaska
- 2007-2008 **Assistant de recherche** de Scott France : projet "Cnidarian Tree of Life"; extractions, PCR et séquençage de l'ADN de coraux de grandes profondeurs
- 2006 **Assistant de recherche** de Jack diTullio (College of Charleston) : Mise en place d'un système d'expérimentation pour l'étude de la production de diméthylsulfure (DMS) chez les zooxanthelles associées aux coraux
- 2004 **Stagiaire**; Centre de Recherche sur les Mammifères Marins, Laboratoire de Biologie et de l'Environnement Marins, Université de La Rochelle. Mai à Juillet. Extraction et amplification ADN
- 2003 **Stagiaire**; Laboratoire de Biologie Marine et Malacologie, École Pratique des Hautes Études, Université de Perpignan (EPHE-UMR CNRS 8046 "Récifs Coralliens"). Février à juillet. Préparation d'un manuscrit sur la zonation des communautés benthiques dans les lagons d'atolls dans l'archipel des Tuamotu
- 2002 **Projet de recherche**. Détection des premiers stades d'infection du parasite ancanthocéphale *Plagiorhynchus cylindrasceus* chez l'isopode *Armadillidium vulgare* à partir de marqueurs moléculaires
- 2002 **Plongeur volontaire**, South Carolina Aquarium (Charleston, SC)
- 2001-02 **Assistant de laboratoire**, Département de Biologie, College of Charleston

Travaux de service communautaire

- Éditeur pour Zootaxa, groupe des octocoralliaires 2012-2016 (<http://www.mapress.com/zootaxa/taxa/Coelenterata.html>)
- Review Editor pour *Frontiers in Marine Systematics and Taxonomy* depuis 2014 (http://www.frontiersin.org/Marine_Systematics_and_Taxonomy/editorialboard)
- Évaluation de manuscrits: <https://publons.com/author/652624/eric-pante>
- 2010 Traduction du blog de vulgarization scientifique de la campagne BioPapua, du français à l'anglais : <http://en.ird.fr/all-the-current-events/live-science/biopapua-cruise>
- 2007 Webmestre du blog de l'École Thématique en Écologie Tropicale de Madagascar
- 2007 Président du comité de préparation du 8ème Symposium des Doctorants de l'Université de Louisiane à Lafayette (financement, organisation)

Activités associatives

Je suis ou étais membre des sociétés suivantes (* par nomination):

- The European Society for Evolutionary Biology
- L'Association Française pour les Récifs Coralliens (French association for coral reefs)
- La Société Française d'Écologie

- The International Biogeography Society
- The Society for Integrative and Comparative Biology
- The Association of American Geographers
- The American Society of Limnology and Oceanography
- The American Association for the Advancement of Science *
- The Sigma Xi Research Society *

Divers

- Citoyen français.
- Français : langue natale. Anglais : parlé et écrit (séjour aux États-Unis d'environ 10 ans)
- analyse de données de séquençage Roche 454 et Illumina HiSeq2000 et MiSeq
- génotypage Illumina BeadXpress
- maîtrise des séquenceurs ABI 3100 and 3130*xl*
- maîtrise de l'environnement de programmation et de statistiques R
- développement du package R **marmap** pour l'analyse des données bathymétriques
- maîtrise des environnements Mac OS X, Windows et Linux
- CMAS plongeur niveau 2; PADI Advanced Open Water diver; South Carolina DNR scientific diver, environ 150 plongées

Quelques références académiques

- Pr. Pascale Garcia ([pgarciam\(at\)univ-lr\(dot\)fr](mailto:pgarciam@univ-lr.fr)), *Responsable de post-doctorat*
Professeure de Biologie et Vice-Présidente du Conseil Scientifique de l'université
Laboratoire LIENSS, Université de La Rochelle, La Rochelle (France)
- Dr. Scott France ([france\(at\)louisiana\(dot\)edu](mailto:france@louisiana.edu)), *Directeur de thèse de doctorat*
Associate Professor et Graduate Admissions Chair
Department of Biology, University of Louisiana at Lafayette, Lafayette LA (USA)
- Pr. Les Watling ([watling\(at\)hawaii\(dot\)edu](mailto:watling@hawaii.edu)), *Rapporteur de la thèse de doctorat*
Professor of Biological Oceanography et Pew Fellow of Marine Conservation
Department of Biology, University of Hawaii at Manoa, Honolulu (USA)
- Pr. Phil Dustan ([dustanp\(at\)cofc\(dot\)edu](mailto:dustanp@cofc.edu)), *Directeur de thèse de master*
Professor of Biological Sciences et Fellow of the Linnean Society
Department of Biology, College of Charleston, Charleston SC (USA)

REGULAR ISSUE FEATURE

LEFT | A freshly collected specimen of the spider crab *Oxypleurodon christiani*, newly described from the BioPapua collections (Richer de Forges and Corbari, 2012). TOP AND BOTTOM | Specimens of king crabs preliminarily attributed to the genus *Paralomis*.

Exploration of the Deep-Sea Fauna of Papua New Guinea

BY ERIC PANTE,
LAURE CORBARI,
JUSTINE THUBAUT,
TIN-YAM CHAN,
RALPH MANA,
MARIE-CATHERINE BOISSELIER,
PHILIPPE BOUCHET,
AND SARAH SAMADI

ABSTRACT. Little is known of New Guinea's deep benthic communities. In fall 2010, the Muséum national d'Histoire naturelle, Institut de Recherche pour le Développement, and University of Papua New Guinea spearheaded an international three-leg cruise, BioPapua, aimed at exploring the deep waters of eastern Papua New Guinea and its satellite islands. Special attention was given to faunal assemblages associated with sunken wood and decomposing vegetation as well as seamount summits and slopes. In this article, we review the information available on the deep ecosystems of Papua New Guinea and summarize preliminary results of the BioPapua cruise.

NEW GUINEA AND OUR UNDERSTANDING OF BIODIVERSITY

The island of New Guinea has a special place in the heart of biologists. Biodiversity studies in New Guinea and its satellite islands have helped shape some of the pillars of evolutionary biology and ecological theory. In the late 1850s, New Guinea was the eastern-most of Alfred Wallace's destinations during his exploration of the Malay Archipelago, and his comparative observations nurtured his theories on island biogeography and the mechanisms promoting evolution (Wallace, 1869, 1876). In the 1920s, ornithologist Ernst Mayr accumulated data on New Guinea birds that would later appear in his *Systematics and the Origin of Species*, a landmark in the development of the modern synthesis (Mayr, 1942). Similarly, data from New Guinean ant diversity found their place in MacArthur and Wilson's theory of island biogeography (MacArthur and Wilson, 1967). Jared Diamond's observations of the bird fauna of New Guinea and its satellite islands nourished his work on the structure of ecological communities and fed the "Single Large Or Several Small [SLOSS]" debate on the application of the principles of biogeography in reserve design and conservation biology (Diamond, 1972, 1973,

1975). More recently, research conducted in New Guinea by Vojtech Novotný and collaborators on plant-insect host specificity has bearing on global biodiversity numbers (e.g., Novotný et al., 2002; Hamilton et al., 2010). And then, of course, there is Tim Flannery's immensely popular *Throwim Way Leg*, a travelogue that perpetuates our perception of New Guinea as a frontier of biodiversity exploration (Flannery, 1998). The faunal diversity of the island's terrestrial, shallow marine, and coral environments is well known; the island is nestled in the middle of the Coral Triangle, a zone delineated by Taiwan, Bali, and the Solomon Islands that is home to about three quarters of the world's coral species (Veron et al., 2009).

In contrast, there is a surprising lack of information on the deep-sea ecosystems surrounding New Guinea and its satellite islands. Symptomatically, the *Challenger* expedition sailed almost directly from Torres Strait to Hong Kong and the Philippines; though it visited the Admiralty Islands in the Bismarck Sea from March 4–10, 1875, only a single deepwater haul was made in 150 fathoms; likewise, the Dutch *Siboga* expedition (1899–1900) did not sample east of Halmahera. In fact, of the "historical" exploring expeditions, only the Danish *Galathea* (1950–1952) and the Russian

Vityaz expeditions collected biological benthic samples in the region, namely the hadal part of the New Britain Trench in the Solomon Sea (Belyaev, 1972).

In fall 2010, the Muséum national d'Histoire naturelle (MNHN) and the Institut de Recherche pour le Développement (IRD; France), in collaboration with the University of Papua New Guinea (UPNG), put together a team of international scientists to explore and describe the deep benthic fauna of the Bismarck and Solomon Seas. The research cruise BioPapua mobilized 11 scientists from six countries, who occupied 156 stations within 2–9°S and 144–155°E (Figure 1). In this article, we review the available information on the deep-sea environments of Papua New Guinea (PNG), and describe the efforts deployed for the BioPapua cruise.

THE DEEP-SEA ENVIRONMENTS OF PAPUA NEW GUINEA

PNG is situated in one of the most geologically active regions in the world, where all possible types of plate boundaries can be observed (Tregoning et al., 2000). Most of the tectonic energy results from the collision of the Indo-Australian and Pacific Plates, which entrap the Bismarck, Solomon, and Woodlark Plates (e.g., Tregoning et al., 2000; Hall, 2002). To the north, the Manus Basin

The authors wish to dedicate this feature to Christian Késiano Fitialeata, who tragically passed away during the first leg of BioPapua. Christian had been a crew member on R/V Alis for many years, and was instrumental in the success of many cruises of the Tropical Deep-Sea Benthos program. He was a great companion at sea and on land, and is sorely missed.

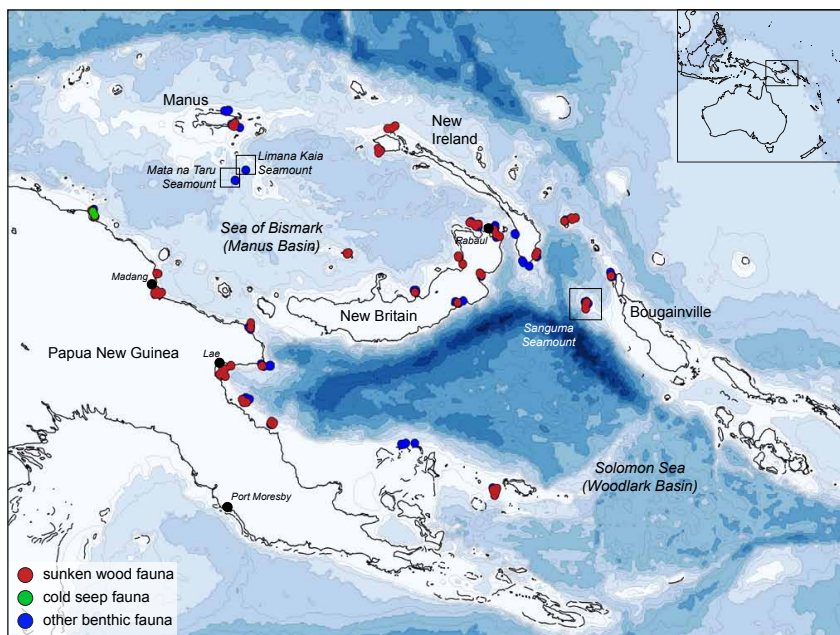


Figure 1. Map of Papua New Guinea and its satellite islands. Dots, color-coded based on faunal type, represent sampling stations. The inset provides a regional map. A marine geological map of PNG can be found in Tregoning et al. (2000).

recently formed (< 4 million years ago) in the Bismarck Sea. This sedimentary basin is characterized by several active accretion zones (the Manus Spreading Center, the South Eastern Rift, and the Western Rift zones) and is rapidly spreading. To the south, the young Woodlark Basin (formed < 2.5 million years ago) produces new oceanic crust, while the Solomon Plate subducts beneath the Bismarck Plate, forming the New Britain chain.

Most of the biological deep-sea scientific exploration done to date in the PNG region stemmed from geological research. PNG's strong tectonic activity is particularly conducive to hydrothermal venting, which was first revealed in the mid- to late 1980s (Both et al., 1986; Tufar 1990; Lisitsyn et al., 1993). A series of international research efforts followed, with cruises involving American, Canadian, Australian, Japanese, Russian,

German, Austrian, and French teams (Auzende et al., 2000, and references therein). These efforts mostly focused on the Pacmanus and Vienna Woods vent fields in the Bismarck Sea, and on Franklin Seamount in the Solomon Sea.

The geological complexity and the rapidity of change observed in the area are of particular biogeographic interest, both in terms of biological colonization and diversity of habitat (e.g., hydrothermal vent fields, river sedimentary cones, seamounts, slopes and canyons, sedimentary plains). Significant efforts have been made to describe the endemic hydrothermal-vent fauna (e.g., Desbruyères et al., 2006, and references therein), but the general deep benthic fauna have yet to be described. For example, the Ocean Biogeographic Information System (OBIS) database contains information on 34 species of deepwater crustaceans, 30 of which are

vent endemic, a very meager inventory for an exclusive economic zone (EEZ) totaling over 2 million km² of benthic surface below 100 m depth (Vanden Berghe, 2007; Edward Vanden Berghe, OBIS, *pers. comm.*, 2011). The Bismarck and Solomon Seas host many underwater features such as seamounts, of which only a very few (e.g., Franklin and Edison Seamounts) have been sampled, mapped, or even visited. Allain et al. (2008) recently reviewed the seamounts of the southwestern Pacific by screening and cross checking 20 existing data sets, and listed 91 underwater features for the EEZ of PNG. No information is available for 69 of those features. The 22 other features were further classified into seamounts (11), drowned atolls and banks (7 and 1), ridges (2), and a plateau. This lack of information on PNG seamounts is reflected by the fact that no seamount site is available on the Seamount Online or Seamount Catalogue databases, not even the few that have been sampled.

NEW GUINEAN DEEP BENTHIC FAUNA IN A REGIONAL BIOGEOGRAPHIC CONTEXT

The current body of knowledge on New Guinean deep-sea benthic fauna comes almost exclusively from the study of highly specialized hydrothermal vent animals, the distribution of which strictly depends on the location of vent fields. Hydrothermal vent animals might therefore not be good models to represent the biology of deep New Guinean fauna. Data on other benthic groups, however, are sorely lacking, and do not allow us to understand patterns of faunal connectivity across the deep waters of the western Pacific Ocean at this time. This section summarizes

current knowledge on the biogeography of deep-sea benthic organisms in the western Pacific Ocean.

Studies of the global biogeography of hydrothermal vent fauna place New Guinean assemblages within a Southwestern Pacific Province. In the analysis of Bachraty et al. (2009), the Mariana back-arc basin, Loihi Seamount offshore Hawaii, seamounts of the Kermadec Arc in New Zealand, and the Central Indian Ridge delimit this region. Previous studies offer different boundaries for this province (e.g., Van Dover et al., 2002; Tyler and Young, 2003) but show faunal affinities between New Guinean vents and the Mariana, North Fiji, and Lau back-arc basins. These affinities, however, are relatively weak, as most vents are characterized by high endemism at the species level. In a multivariate community analysis, Desbruyères et al. (2006) found that the highest similarity level between vents of the Manus basin and other Southwest Pacific locations was 24% at the species level and 46% at the genus level, and linked the New Guinean vents to the communities of the North Fiji and Lau back-arc basins. In fact, Vrijenhoek (2010) and Van Dover (2011) recently proposed a biogeographic model in which New Guinean vents constitute the northwestern end of the range of a distinct Southwestern Pacific Province. The New Guinean back-arc basins are particularly isolated within the mosaic of western Pacific spreading centers, as about 1,500 km separate the Woodlark Basin from North Fiji and Manus from the Mariana Basin (Hessler and Lonsdale, 1991).

The geographic isolation of PNG hydrothermal-vent organisms may have

strongly affected their evolutionary history and current distribution. This fauna may therefore not be representative of the overall biogeography of deep-sea benthic fauna from this area. Unfortunately, data from other deep-sea organisms are very scarce. The known biogeography of the deep-sea coral genus *Chrysogorgia* well exemplifies the lack of information on nonvent, deep benthic fauna (Figure 2; Pante et al., 2012). *Chrysogorgia* shows sampling gaps around PNG indicative of our state of knowledge on the New Guinean deep-sea fauna. Out of 65 species (and variations), 21 were originally described from locations in or on the immediate periphery of the Coral Triangle. Although five species from the Malay Archipelago were also observed in the Northeast Pacific (Hawaiian Archipelago), only two species were found to occur both east and west of the island of New Guinea. Although these results might simply be a sampling artifact or the result of incomplete taxonomic knowledge, they might also reflect actual biogeographic patterns.

Other invertebrate models suggest a dichotomy between northern and southern biogeographic provinces. In a large-scale analysis of squat lobster biogeographic patterns, Macpherson et al. (2010) established that the fauna of the Coral Sea (New Caledonia, Vanuatu, and the Solomon Islands) was significantly different from the species assemblages found in eastern Australia, Indonesia (Banda and Celebes Seas), and the Philippines. No data were available for PNG. Similar biogeographic studies focusing on deep-sea bathymodioline mussels support the same overall geographic separations, with mussel assemblages being very similar among New Caledonia, Vanuatu, and the Solomon Islands, and different from northern locations such as the Philippines (Lorion et al., 2010; Lorion and Samadi, 2010). Again, no data were available for PNG. Puillandre et al. (2010), questioning how modes of larval dispersal relate to geographical fragmentation in the gastropod *Bathytoma* in the western Pacific, also underlined the sore lack of

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specimens from New Guinea.

It is clear from hydrothermal vent community data available today, as well as from the biogeographic patterns of corals, galatheid crabs, and bathymodioline mussels, that the deep waters of PNG are located at the confluence of different biogeographic regions. The foundations of our exploration of PNG deep benthic ecosystems are therefore both discovery- and hypothesis-driven, as we aim to document the deepwater benthic organisms present in nonhydrothermal ecosystems and to understand their historical and contemporary biogeography in the context of the tropical Western Pacific. Are organisms from this region more closely related to southern, northern, or western species pools? What are

the historical and contemporary mechanisms leading to the faunal discontinuities within the region?

BIOPAPUA AND THE TROPICAL DEEP-SEA BENTHOS PROGRAM

BioPapua is part of a long-term research endeavor called the Tropical Deep-Sea Benthos (TDSB) program. Started in the 1980s by the IRD (formerly Office de la Recherche Scientifique et Technique Outre-Mer [ORSTOM]) and the MNHN, its goal is to explore the deep benthic ecosystems of the tropical Indo-West Pacific, with special emphasis on macroinvertebrates. Since the late 1970s, more than 60 TDSB cruises have been conducted from Taiwan to the Marquesas Islands, and sampling was

conducted at over 5,000 stations between 100 and 1,500 m depth. Through taxonomic networking with an international team of more than 200 scientists, over 2,000 new species have been described based on material from the TDSB program (Bouchet et al., 2008).

TDSB cruises, because of their robustness and relative technical simplicity, often represent the first-ever exploration of the deep-sea benthos in remote areas such as Tonga, the Solomon Islands, or Vanuatu. BioPapua, as most TDSB cruises, relied on the New Caledonia-based R/V *Alis*, a 28 m long ship with high maneuverability. The combined use of a beam trawl and a dredge maximizes the types of substrates and faunal diversity that can be collected (Figure 3).

BIOPAPUA RESEARCH THEMES AND PRELIMINARY RESULTS

The first purpose of BioPapua, as for previous TDSB cruises, was to explore remote and uncharted territory and describe its deep benthic fauna. Beyond this effort of discovery, BioPapua was designed to sample specimens from targeted habitats and address specific questions on the evolution of deep-water invertebrates. We outline research themes and preliminary results below.

Sunken Wood and the Evolution of Chemosynthetic Organisms

Since their discovery in 1977 on the Galápagos Spreading Center (Lonsdale, 1977), hydrothermal vents have been the focus of tremendous research interest. Although significant efforts were put forth to characterize this environment and elucidate the mechanisms involved in its functioning and maintenance, much less is

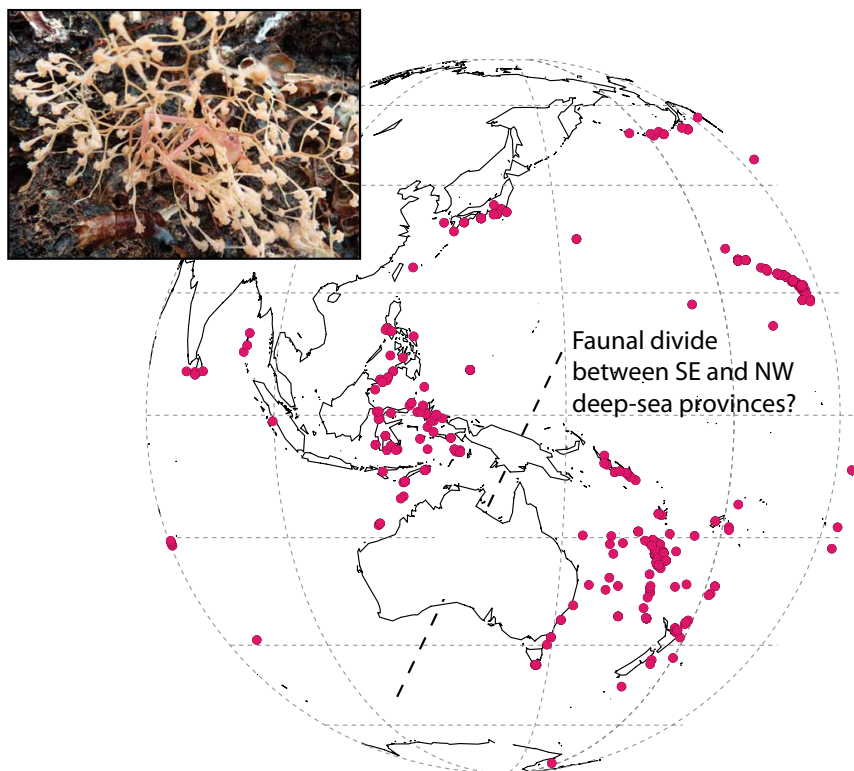


Figure 2. Geographic distribution of 634 records of the octocoral genus *Chrysogorgia*. Inset: one of the 56 colonies of *Chrysogorgia* sampled during BioPapua, with associated squat lobster (data from Watling et al., 2011).

known about the evolutionary origins of its fauna. Phylogenetic studies of the deep-sea mussels of the subfamily Bathymodiolinae show that species from vents, seeps, and deep-sea organic falls (vegetation and whale carcasses) form monophyletic clades, suggesting that these deep-sea bivalves may have used organic falls as a transitional habitat from shallow habitats to deep vents and seeps (Distel et al., 2000). Recent results suggest that the evolutionary history of deep-sea mussels associated with organic falls is completely tangled with that of vent and seep mussels, with scenarios probably more complex than the “wooden steps” hypothesis (Lorion et al., 2010). Moreover, geography, rather than habitat specificity or chemosymbiotic requirements, seems to significantly structure diversification patterns in sunken wood-associated mytilids (Lorion et al., 2009, 2010).

There are still large gaps in our understanding of the series of evolutionary events that lead to the diversification of vent, seep, and organic fall fauna. First, sampling plays a crucial role in comparative phylogenetic inference, and most studies to date are biased toward a more robust sampling of vents and seeps. Incomplete sampling can lead to phylogenies that do not accurately reflect evolutionary history. There is therefore a need to thoroughly characterize both vent/seep and organic fall biodiversity. One objective of BioPapua was to provide new material that may promote understanding of the evolutionary origins of the crustaceans and mollusks inhabiting deep-sea chemosynthetic environments. To fill the identified sampling gaps, the main objectives are (1) to describe the

diversity (morphological, genetic, and ecological) associated with organic falls, (2) to determine how organisms rely on organic remains, and (3) to investigate the historical and ecological factors that may have influenced the diversification of this fauna.

Among the 156 stations sampled during BioPapua, 84 contained sunken vegetation. A wide array of bays and submarine canyons was sampled, from the mouth of the Sepik River to Mambare Bay on the eastern coast of the island of New Guinea, and on the eastern and western coasts of New Britain. Trawling revealed a wealth of decomposing wild and cultivated plants (tree trunks, branches and leaves, sugar cane, coco, and nypa nuts). Decaying vegetation was found accumulating at the bottom of submarine canyons. As expected, these substrates were host to

a unique fauna, composed of mollusks (wood-boring bivalves and mytilids, chitons, gastropods; see Warén, 2011), crustaceans (isopods, amphipods, barnacles, and decapods), echinoderms (sea urchins, sea stars, and ophiuroids), polychaetes (19 families identified so far), and sipunculids (see Figure B1 in Box 1), a composition that was consistent with previous findings from other locations in the Southwest Pacific (Samadi et al., 2010). For example, the crustaceans collected on plant remains belong to a few genera repeatedly found on such material throughout the Indo-Pacific (for decapods: the galatheids *Munida* and *Munidopsis*, the hermit crabs *Xylopagurus* and *Pylocheles*, and the thalassinids *Callianassa*; Samadi et al., 2010). Most of the taxa, however, probably belong to new species. Author Thubaut and colleagues included nine

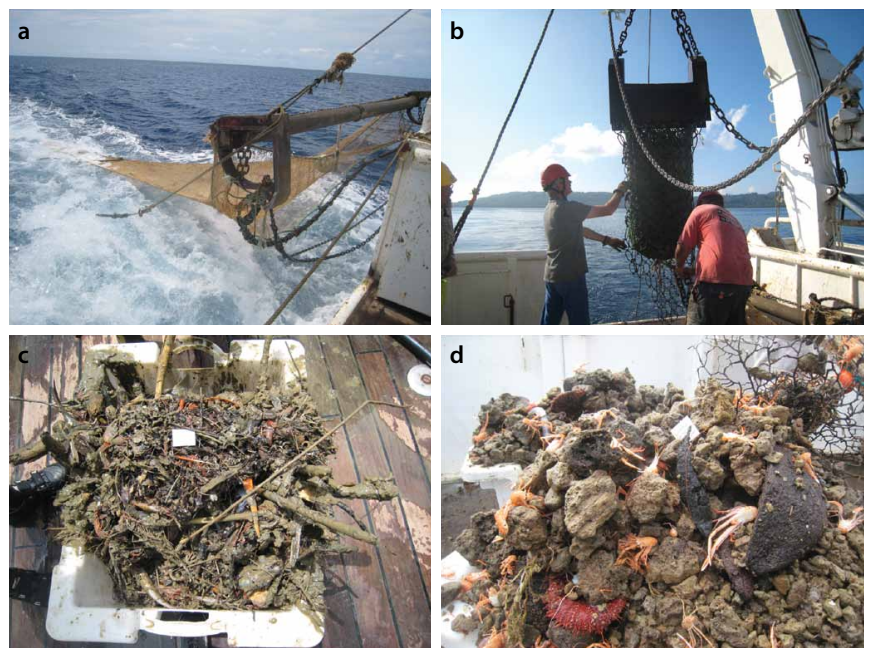


Figure 3. Sampling strategy employed during BioPapua and other Tropical Deep-Sea Benthos cruises. (a) Beam trawl. (b) Warén dredge. (c–d) Raw samples that illustrate the abundance of fauna collected and the diversity of substrates.

BOX 1. VEGETATION-ASSOCIATED FAUNA

The *Challenger* expedition (1872–1876) casually documented the presence of animals on plant remains at the deep seafloor, but the importance of plant material in the deep sea was not underscored until Wolff (1979). Until recently, plant-associated organisms were mainly looked at as zoological and/or ecological curiosities and thus (apart from taxonomic consideration) only anecdotally studied. During previous TDSB cruises in the Solomon Islands, it became clear that the slopes of oceanic islands within the region accumulate large amounts of decomposing vegetation. Organic debris was associated with a rich and original fauna (Samadi et al., 2007a; Warén, 2011), and its discovery catalyzed new research interests for the TDSB team (Samadi et al., 2010). Subsequent cruises in the Solomon Islands and Vanuatu specifically targeted sunken vegetation to further characterize this fauna. PNG and its satellite islands appear to be a very promising location for such studies. Their lush coastal tropical forests and large rivers transition into bays and canyons that may accumulate sunken vegetation. Figure B1 shows some of the diversity found on plant remains. *Adipicola longissima* (Figure B1 c) is a mussel found mainly in association with *Nypa fruticans* nuts. In contrast with other sunken wood mussels that have extensive geographic distributions in the Pacific, *Adipicola longissima* is restricted to the areas where nypa palms grow (mainly the Philippines, Indonesia, PNG, and the northern area of the Solomon Islands). Chitons revealed unsuspected diversity, and a wealth of undescribed species (Figure B1 a–f; Nicolas Puillandre, Muséum national d'Histoire naturelle, pers. comm., 2012). The amphipod genus *Bathyceradocus* (Maeridae; Figure B1 i) was represented by eight species, four of them new. A wood-based diet and the presence of

bacterial ectosymbiosis have been confirmed for several specimens, and molecular analyses revealed unexpectedly high diversity (recent work of author Corbari). Wood-dependent diets and bacterial interactions have already been demonstrated for different wood-associated organisms (sea urchins, Becker et al., 2009; *Pectinodonta*, Zbinden et al., 2010; *Munidopsidae*, Hoyoux et al., 2009). Future work will focus on placing trophic relationships in an evolutionary context.

Animals are not the only active decomposers of plant remains. Wood decay also results from the metabolic activities of bacteria (Waterbury et al., 1983; Distel and Roberts, 1997) and fungi (Ray, 1959; Ray and Stuntz, 1959). Although shallow marine fungi are common and diverse (550 species described, at least 1,500 expected to exist), deep-sea fungi appear to be rare (four species described from > 1,600 m) and are still little studied (Dupont et al., 2009, and references therein). For example, *Oceanitis scuticella*, originally described in 1977, was not seen until 2004, when specimens were collected in Vanuatu and the Solomon Islands during TDSB cruises (Dupont et al., 2009). *Alisea longicolla*, a new genus and species described from material collected alongside *O. scuticella* during a 2005 TDSB cruise (Dupont et al., 2009), is another example of how rare and understudied taxa are brought to light by the exploratory TDSB cruises. During BioPapua, deep-sea fungi were sampled at seven stations deeper than 700 m. Samples include a new occurrence for *O. scuticella* and also a new occurrence (at 700–1,150 m depth) of an undescribed morpho-species that was previously known from only a few specimens collected in 2007 off the Solomon Islands (Figure B1 g).

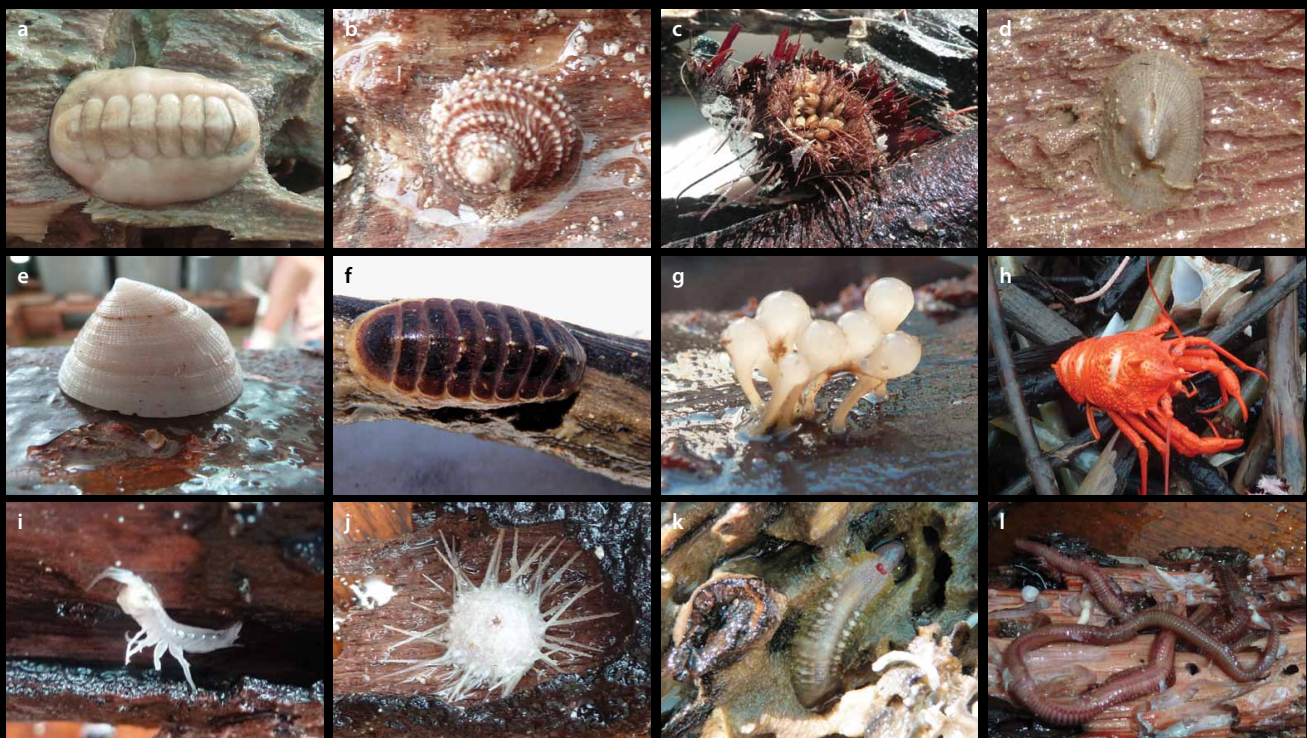


Figure B1. Deck photos of vegetation-associated fauna. (a) Chiton, *Ferreiraella* sp. (b) Cataegidae, *Cataegis* sp. (c) Mytilidae, *Adipicola longissima* nested in nypa palm nut. (d) Fissurellidae, *Puncturella* sp. (e) Pectinodontidae, *Pectinodonta* sp. (f) Chiton *Leptochiton* sp. (g) Undescribed fungi. (h) Munidopsidae *Galacantha* sp. (i) Maeridae, *Bathyceradocus* sp. (j) ?Echinidae. (k) Polynoidae, *Harmothoe* sp. (l) Eunicidae, *Eunice* sp.

samples collected during BioPapua into a larger study of the biogeography and integrative biology of *Adipicola iwaotakii*, a small bathymodioline mussel associated with sunken wood. This group has found high genetic diversity (seven mitochondrial COI haplotypes), a signal of population expansion, and a closer biogeographic relationship to the Solomon Islands, Vanuatu, and New Caledonia than to Japan and the Philippines. Among the 26 putative species of wood-associated mussels recognized by Lorion et al. (2010) for the Southwest Pacific, 11 were collected during BioPapua. None of the nine putative species found in the Philippines and Japan were collected in PNG; the wood-associated mussel fauna of PNG has therefore stronger affinities with southern locations such as New Caledonia.

Connectivity and Endemism of Seamount Fauna

Describing biodiversity from underwater mountains or seamounts (e.g., Stocks, 2009) has become one of the major themes of deep-sea exploration (e.g., special issue on “Mountains in the Sea,” *Oceanography* vol. 23, March 2010, <http://www.tos.org/oceanography/archive/23-1.html>). Seamounts are among the most ubiquitous underwater features in the ocean (Wessel et al., 2010) and may constitute one of the largest marine biomes, with a cumulative surface area exceeding 17 million km² (Etnoyer et al., 2010; Yesson et al., 2011).

Seamounts constitute an ideal system for the study of deep-sea biogeography as they are prevalent across ocean basins, are patches of hard substrata scattered on vast sedimentary plains, and are characterized by hydrographic

features that could significantly hinder faunal dispersal (e.g., Clark et al., 2010). The extent of faunal connectivity among seamounts and seamount chains is, however, still poorly known. Beyond theoretical inquiries, there is a strong practical need to understand seamount ecosystems in order to manage fragile fisheries resources and associated fauna. Seamounts aggregate commercially important fish species and are hotspots of pelagic and benthic biodiversity (Samadi et al., 2007b; Morato et al., 2010). The impact of deep-sea trawling on developing seamount fisheries has been shown to be catastrophic and long lasting (Althaus et al., 2009; Williams et al., 2010); therefore, there is no more critical time to study seamount ecosystems.

The scarcity of data on seamount fauna, however, prevents us from conceptualizing the processes that shape and maintain seamount faunal assemblages. The major obstacles to describing the biodiversity and biogeography of seamount fauna are (1) a lack of geographically comprehensive sampling, (2) the paucity of model systems allowing faunal comparisons across seamount chains, oceans, and depth regimes, and (3) the lack of integration of genetic information into the estimation of endemism levels. Indeed, since the inception of seamount science, many paradigms for the ecology of seamount fauna have been proposed, but few are strongly supported by empirical evidence (Rowden et al., 2010). There is therefore a strong need for the seamount research community to sample across the diversity of seamount environments (e.g., depth range, steepness, geological origin, habitat composition, and water-column

productivity) and geographical locations (e.g., latitude and isolation from continental margins).

One of the goals of BioPapua was to help fill the geographical gap in our understanding of Pacific seamount communities. Three of the seamounts catalogued by Allain et al. (2008) were sampled during BioPapua. Two are located in the Bismarck Sea, between Manus Island and the Western Rift accretion zone (3.05°S, 147.53°E and 3.23°S, 147.33°E, five stations sampled). The first seamount, Limana Kaia, appeared poor in benthic fauna, as our trawl mostly brought back bare rocks. This negative result might be explained by limited and/or unsuccessful sampling on rugged terrain. The second seamount, Mata Na Taru, is more interesting from a biological standpoint, as we were able to collect a diverse fauna. The third seamount (Sanguma, 5.53°S, 154°E, seven stations sampled) is located east of the north end of Bougainville Island, on the edge of the Solomon Trench, an active zone of subduction of the Solomon Plate under the Pacific Plate. This seamount, sampled at depths between 369 and 768 m, revealed a wealth of filter-feeding invertebrates, such as hydroids, sea pens, octocorals, and sea anemones, as well as a rich crustacean fauna.

Forty-four live specimens of *Nassaria* were collected during BioPapua, including seven on Sanguma Seamount. This neogastropod, with a nonplanktotrophic, poorly dispersive larval stage, is one of the model systems used to investigate faunal connectivity patterns between slope and seamount environments in New Caledonia (Castelin, 2010; Castelin et al., 2010). Among the 250 individuals previously analyzed (collected during

seven cruises representing approximately 800 sampling stations), 11 putative molecular species were detected, of which four were found exclusively in the Philippines (111 stations), and seven in New Caledonia (600 stations). Among the 44 BioPapua specimens of *Nassaria*, nine putative molecular species (one

common among BioPapua stations, all the others being rare) were detected, and only two of these are shared with the rich New Caledonian fauna (Magalie Castelin, Muséum national d'Histoire naturelle, *pers. comm.*, 2012).

Among octocorals, *Chrysogorgia* was particularly diverse, as 12 putative

species (based on DNA barcoding using *mtMutS*; McFadden et al., 2010; Pante and Watling, 2011) were detected, 25% of them new to our collections (Pante et al., 2012) and most being rare. This species pool represents 35% of the diversity found in the Pacific, and 27% of the total haplotypic richness of

BOX 2. BIODIVERSITY HIGHLIGHTS

One main focus of TDSB cruises is to explore and document deep-sea biodiversity. While searching for accumulations of sunken vegetation at the mouth of the Sepik River (03°53'S, 144°41'E), we sampled organisms typically found at cold seeps and hydrothermal vents, among them beard worms (Siboglinidae: *Escarpia* sp. and *Lamellibrachia* sp.) and two undescribed species of bathymodioline mussels (*Gigantidas* sp.; Figure B2 e–f), one of which associated with a polynoid scale worm (*Branchipolynoe* aff. *pettiboneae*; Figure B2 g). Several undescribed species of *Gigantidas* were already known from nearby seep localities (Kyuno et al., 2009) off New Guinea. On the last leg of BioPapua, we sampled the extraordinary copepod *Cardiodectes* sp. (Figure B2 i; Jean-Lou Justine, Muséum national d'Histoire naturelle, *pers. comm.*, 2012) on the eye of its fish host, and eulimid gastropods neatly attached on their urchin host (Figure B2 c). Species of the Eulimidae are all parasites of echinoderms, and much work remains to be done to understand patterns of host specificity and echinoderm-mollusk co-evolution. Another noteworthy find was a specimen of *Bayerotrochus* (Figure B2 a). This grazing mollusk of the family Pleurotomariidae has a continuous fossil record since the Silurian. As for many other groups, pleurotomariids had never before been recorded from the vicinity of New Guinea (Harasewych, 2002). This specimen probably belongs to an undescribed species (Patrick Anseeuw, Royal Belgian Institute of Natural Sciences, *pers. comm.*, 2012).



Figure B2. Biodiversity examples: (a) Pleurotomariidae, *Bayerotrochus* sp. (b) Amphinomidae, *Chloeia* sp. (c) Eulimidae, *Pelseneeria* sp. on sea-urchin. (d) Nassariidae, *Nassarius* sp. (e) Mytilidae, *Gigantidas* sp. nov. 1. (f–g) Mytilidae, *Gigantidas* sp. nov. 2 in association with *Branchipolynoe* sp. nov. (h) Buccinidae, *Nassaria* sp. (i) Parasitic copepod Pennellidae, *Cardiodectes* sp. (j) Deep-sea fish, ?Chauliodidae. (k) Cirripedia, Lepadidae.

the genus (although we warn that these results will have to be analyzed using rarefaction analysis). Interestingly, two of these 12 putative species collected on Sanguma Seamount were also previously found on New Caledonian seamounts (one on Norfolk Ridge, the other on Loyalty Ridge), suggesting, as for the gastropod *Nassaria*, broad geographical distribution of these seamount organisms.

CONCLUSION

Very little is known of the biodiversity and biogeography of deep-sea animals from PNG. The data available, however, suggest that deep New Guinean fauna is at the confluence of significantly different biogeographic regions, and is more closely related to the southern than the northern Pacific fauna. Preliminary data suggest that deep New Guinean fauna is highly diversified. As an example, a rapid assessment of decapod crustacean biodiversity revealed more than 500 species collected, including four new genera (in the families Solenoceridae, Galatheidae, Callianassidae and Parapaguridae; Figure 4) and about 15% new species. We expect that further study of the material collected during BioPapua will shed light on large-scale biogeographic patterns in the tropical deep sea of the Western Pacific Ocean. While we are just beginning to fathom the amplitude of biodiversity in deep New Guinean waters, mining exploration and exploitation of seafloor massive sulfides (SMS) is flourishing in the region (Hoagland et al., 2010; Van Dover, 2011). The exploitation of metals from SMS deposits is an emerging industry, and adverse environmental effects are hard to predict, as hydrothermal vent and adjacent




Figure 4. New and undescribed crustacean species. (a–b) Family Solenoceridae, *Maximiliaeus odoceros* (newly described by Chan, 2012). (c) Family Epialtidae, *Oxypleurodon stimpsoni*. (d) Family Goneplacidae, *Thyraplax* sp. (e) Family Paguridae, *Bathypaguropsis* sp. (f) Family Munidopsidae, *Munidopsis* sp. Photos: Tin-Yam Chan

ecosystems are still poorly known. We therefore hope that our geographically comprehensive sampling program will provide a baseline for the biodiversity of the deep benthic organisms of the Bismarck and Solomon Seas.

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ORIGINAL ARTICLE

Use of RAD sequencing for delimiting species

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RAD-tag sequencing is a promising method for conducting genome-wide evolutionary studies. However, to date, only a handful of studies empirically tested its applicability above the species level. In this communication, we use RAD tags to contribute to the delimitation of species within a diverse genus of deep-sea octocorals, *Chrysogorgia*, for which few classical genetic markers have proved informative. Previous studies have hypothesized that single mitochondrial haplotypes can be used to delimit *Chrysogorgia* species. On the basis of two lanes of Illumina sequencing, we inferred phylogenetic relationships among 12 putative species that were delimited using mitochondrial data, comparing two RAD analysis pipelines (Stacks and PyRAD). The number of homologous RAD loci decreased dramatically with increasing divergence, as >70% of loci are lost when comparing specimens separated by two mutations on the 700-nt long mitochondrial phylogeny. Species delimitation hypotheses based on the mitochondrial *mtMutS* gene are largely supported, as six out of nine putative species represented by more than one colony were recovered as discrete, well-supported clades. Significant genetic structure (correlating with geography) was detected within one putative species, suggesting that individuals characterized by the same *mtMutS* haplotype may belong to distinct species. Conversely, three *mtMutS* haplotypes formed one well-supported clade within which no population structure was detected, also suggesting that intraspecific variation exists at *mtMutS* in *Chrysogorgia*. Despite an impressive decrease in the number of homologous loci across clades, RAD data helped us to fine-tune our interpretations of classical mitochondrial markers used in octocoral species delimitation, and discover previously undetected diversity.

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INTRODUCTION

The advent of next-generation sequencing tools has permitted significant advances in our understanding of evolutionary processes such as speciation (for example, Ekblom and Galindo, 2011), but some other practical applications of genomic data have been less explored, including phylogenomics and species delimitation. Among genomic approaches that are applicable to these fields, the usefulness of restriction site-associated DNA tag (RAD tag; Baird *et al.*, 2008) sequencing has been investigated in few studies to date. This methodology typically provides short sequences (~100–150 bp) flanking the cut sites of a restriction enzyme (or several enzymes), generally yielding thousands of loci distributed throughout the genome. This approach does not require a reference genome and can therefore be applied to non-model organisms. However, some technical difficulties remain for groups where very little genomic knowledge is available (see Davey *et al.*, 2011). For instance, the choice of restriction enzyme (s) and methodology (single-digest versus double-digest RAD) is key to estimating the number of expected cut sites and coverage, but relies on prior knowledge of genome size and GC content.

Despite these difficulties, RAD-tag sequencing constitutes one of the reduced genomic approaches that are suitable for investigating interspecific evolutionary questions. Published RAD-tag sequencing research beyond the species level includes *in silico* studies (*Drosophila*, mammals and yeasts in Rubin *et al.*, 2012; *Drosophila* in Cariou *et al.*,

2013) and empirical work (for example, Restionaceae flowering plants in Lexer *et al.*, 2013; cetaceans in Viricel *et al.*, 2014), which both suggest this approach is promising for taxa having diverged up to 60 million years (Myr) ago. For instance, RAD-tag sequencing has proven useful in species delimitation and phylogenies within recently and rapidly diverged groups (for example, Orobanchaceae flowering plants in Eaton and Ree, 2013; swordtails in Jones *et al.*, 2013; *Heliconius* butterflies in Nadeau *et al.*, 2013; cichlids in Wagner *et al.*, 2013; geckos in Leaché *et al.*, 2014). Comparatively, reconstructing the phylogeny of more distantly related taxa has been the topic of two study (*Carabus* beetles, Cruaud *et al.*, 2014; oak trees, Hipp *et al.*, 2014). Herein, we use this approach on a group of deep-sea octocorals for which little genomic data are available. Thus, our contribution constitutes one of the first studies investigating the use of RAD-tag sequencing for practical species delimitation within a taxonomic group composed of divergent species (up to 16 Myr ago).

Deep-sea octocorals are one of the groups for which RAD-tag sequencing can significantly advance our understanding of evolutionary patterns. As for shallow-water octocorals, deep-water octocorals present significant challenges for taxonomists, with few morphological characters being available for species delimitation (for example, McFadden *et al.*, 2010). In addition, several studies have shown conflicting patterns of morphological and molecular data (France, 2007; Dueñas and Sánchez, 2009; Pante and France, 2010), suggesting

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that an integrative approach to taxon delimitation must be applied in this group (for example, Schlick-Steiner *et al.*, 2010). Octocorals, as with other anthozoans (for example, scleractinians and sea anemones), are also plagued with remarkably low levels of mitochondrial genome evolution that renders the use of classical barcoding gene regions such as *cox1* of limited use (McFadden *et al.*, 2011). Comparatively, a few studies have successfully used nuclear markers within octocoral species (for example, Concepcion *et al.*, 2008; Mokhtar-Jamaï *et al.*, 2011), but these are either not widely useable across octocorals (for example, SRP54; France and Pante, unpublished observations), or not informative at multiple phylogenetic scales (for example, microsatellites). Multicopy markers have been employed (for example, Herrera *et al.*, 2010); however, their use implies that lack of concerted evolution within and across genomes will not blur the phylogenetic signal (Vollmer and Palumbi, 2004; Calderón *et al.*, 2006). In this group, RAD-tag genotyping may therefore offer a panel of markers to help describe patterns of population structure, delimit species and investigate phylogenetic relationships. This technique may however be difficult to implement in this group. Indeed, the composition of the deep-sea octocoral genome is unknown (size, GC content, prevalence of cut sites for restriction enzymes and so on); the size of known cnidarian genomes, for instance, varies between 224 Mb and 1.8 Tb (Animal Genome Size Database; Gregory, 2014). In addition, sampling of deep-sea animals can be associated with a loss of quality of genomic DNA samples, particularly when sampling in tropical waters using trawls or dredges.

The genus *Chrysogorgia* (Calcaxonia: Chrysogorgiidae) is a noteworthy model for testing the utility of RAD sequencing for delimiting octocoral species, as it is diverse (62 nominal species described, 93% of which were based solely on morphology), widely distributed and can be locally abundant (Watling *et al.*, 2011). The large geographic, bathymetric and ecological distributions of some *Chrysogorgia* species (Pante *et al.*, 2012b) question whether taxa are appropriately delimited, and whether cryptic diversity is important in the group. In the northwestern Atlantic, congruence exists between morphological and genetic data, suggesting that a relatively short fragment of the mitochondrial *mtMutS* gene can be used to formulate 'Primary Species Hypotheses' (Pante and Watling, 2012). It is suspected that little to no intraspecific variation exists for this marker within the group (McFadden *et al.*, 2011), but the null hypothesis that single mutations at *mtMutS* are diagnostic of species limits must be evaluated using genetic data from markers informative within and above the species

level. RAD loci allow to test whether lineages that putatively belong to different species do not exchange genes.

In this communication, we test the utility of RAD-tag genotyping for delimiting species in *Chrysogorgia* using the genealogical criterion defined by Taylor *et al.* (2000). More specifically, we test whether single mutations on the mitochondrial *mtMutS* gene can be used as a criterion for grouping *Chrysogorgia* colonies into separate, putative species (or, more specifically, 'Primary Species Delimitation hypotheses' as in Puillandre *et al.*, 2012). We compare the results from two analysis pipelines, Stacks (Catchen *et al.*, 2013) and PyRAD (Eaton, 2014), which significantly differ in the method employed for detecting homologous loci.

MATERIALS AND METHODS

Specimen collection and mtDNA typing

Chrysogorgia specimens were collected from the SE slope of New Caledonia (NC) and adjacent seamounts of the Norfolk Ridge (82 colonies; Terrasses cruise, 2008), from Papua New Guinea (PNG; 8 colonies; BioPapua cruise, 2010) and from the northwestern Atlantic (1 colony, Extreme Coral 2010 cruise; Table 1 and Supplementary Table S1). Pacific specimens were retrieved from dredges and trawls (details on cruises of the Tropical Deep Sea Benthos research program: Bouchet *et al.*, 2008; details on the BioPapua cruise: Pante *et al.*, 2012a); the Atlantic specimen was collected using the Jason II ROV (Woods Hole Oceanographic Institution). Specimens were fixed in 80% ethanol as soon as possible after collection. Genomic DNA was extracted using a CTAB protocol according to France *et al.* (1996). A 700-bp fragment of the mitochondrial *mtMutS* gene (identified as more informative than *cox1* or 18S in chrysogorgiids, Pante *et al.*, 2012b) was amplified using the ND4L2475F–MUT3458R primer pair and sequenced using an ABI PRISM (R) 3100 or 3130xl Genetic Analyzer (primer information, PCR and sequencing conditions: Pante *et al.*, 2012b). Sequences were checked for quality and edited in Sequencher (TM) 4.7 (Gene Codes), aligned by eye (a single, 3-bp indel was present in the alignment) and haplotypes were submitted to GenBank (Supplementary Table S1). Divergence times among putative species were estimated using the molecular clock from Lepard (2003), which was calculated for the shallow-water octocoral genus *Leptogorgia* based on *mtMutS* genetic distances for clades located on either sides of the Isthmus of Panama (0.14–0.25% per Myr).

Library construction, RAD sequencing and quality control

Genomic DNA quality was evaluated by 1% agarose gel electrophoresis and quantified using a Thermo Scientific (Waltham, MA, USA) Nanodrop ND-1000 spectrophotometer. DNA was sent to Eurofins Genomics (Ebersberg, Germany) for RAD-tag library preparation and sequencing. Libraries were

Table 1 Summary table of haplotype information (sample size, geographical spread, depth range, habitat (seamounts vs slopes) and *mtMutS* vs RAD delimitation

Haplotype	No. of colonies	Geography	Habitat	Depth range (m)	Delimitation
J	1	Atlantic	Slope	627–627	mtMutS/RAD congruence
2	11	NC	Slope	390–500	mtMutS/RAD incongruence
4	20	NC	Slope and seamount	150–330	mtMutS/RAD congruence
6	2	NC	Seamount	270–310	mtMutS/RAD congruence
7	8	NC-PNG	Slope and seamount	300–880	mtMutS/RAD incongruence
8	20	NC	Slope	390–500	mtMutS/RAD incongruence
9	18	NC	Slope	390–450	mtMutS/RAD congruence
10	3	NC	Slope and seamount	458–880	mtMutS/RAD congruence
11	3	NC	Seamount	750–840	mtMutS/RAD congruence
13	1	NC	Slope	460–490	mtMutS/RAD incongruence
14	1	NC	Slope	400–420	mtMutS/RAD congruence
30	3	PNG	Slope	220–1020	mtMutS/RAD congruence

Abbreviations: NC, New Caledonia, PNG, Papua New Guinea.

constructed from 1–2 µg of DNA per colony using the *SbfI* restriction enzyme. This enzyme was chosen because it was successfully used in RADseq experiments with marine invertebrates (sea anemones, Reitzel *et al.*, 2013; abalone, Gruenthal *et al.*, 2014), and was expected to allow an acceptable compromise between prevalence of cut sites and depth of coverage, based on RADcounter (the University of Edinburgh, <https://www.wiki.ed.ac.uk/display/RADSequencing/Home>). As the genome size and GC content of *Chrysogorgia* (or other octocorals, to the best of our knowledge) are not known, we estimated the prevalence of *SbfI* cut sites based on a range of genome sizes and GC content, based on information from the Animal Genome Size Database (see Introduction) and with a GC content of 40% (for example, Soza-Ried *et al.*, 2010). Barcodes 6–9 nt long and differing by at least 2 nt were used to differentiate multiplexed samples (Supplementary Table S1). Sequencing was performed on two lanes of the Illumina (R) HiSeq (TM) 2000 instrument (Illumina Inc., San Diego, CA, USA) using the single read, 100 nucleotide configuration. Raw HiSeq output was processed using the CASAVA v1.8.2 software pipeline (Illumina Inc.) and demultiplexed and quality filtered using the process_radtags.pl module (default quality settings) of the Stacks v.0.99994 pipeline (Catchen *et al.*, 2013). A single sequencing error was tolerated in the barcode. Reads were truncated to 91 nt. Quality (as measured by phred scores and percentage of sequence overrepresentation) was checked before and after treatment by process_radtags using FastQC v.0.10.1 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>).

Exploration of the divergence parameter space

Two main pipelines specifically designed for analysis of RADseq data are currently available. The most used to date is the Stacks pipeline. It constructs a catalog of loci for a set of samples mainly based on three parameters: the minimum stack depth parameter *m* (that is, the minimum number of reads allowed per allele), the intraindividual divergence parameter *M* (that is, the maximum number of mutations that can be observed between stacks within a sample), and the interindividual divergence parameter *n* (that is, the maximum number of mutations that can be observed between loci across samples).

PyRAD (Eaton, 2014) is a more recently developed pipeline and differs from Stacks in several ways, the most important one being that it allows the presence of insertions and deletions (indels), as the clustering process of reads into loci uses alignment tools. This is anticipated to be an advantage compared with the first pipeline when considering more phylogenetically distant species. PyRAD relies on a large number of parameters used at different steps of the process. Most of them are related to reads quality control, detection of homology and filtering of paralogs. Two main parameters are of particular importance: the minimum depth coverage Mindepth (minimum depth necessary to make a statistical base call at each position of a cluster) and the similarity threshold Wclust (similarity value to be used for the alignment during both the within and across-sample clustering).

For both pipelines, these parameter settings are expected to influence greatly the number of markers available for intra- and interspecific comparisons and it is necessary to explore which parameter combinations maximize the number of orthologous loci (Viricel *et al.*, 2014). To explore the effect of these parameters at different phylogenetic depths, we randomly selected pairs of specimens that (1) were separated by 0–16 mutations at *mtMutS* (representing different levels of phylogenetic divergence) and (2) were characterized by 1–1.5 million reads (to alleviate potential effects of depth of coverage on the number of assembled loci). For each level of divergence, we used three replicate pairs of specimens. We refer to specimens with *mtMutS* haplotypes differing by few mutations as pairs of closely related colonies, and those with haplotypes differing by many mutations as distantly related colonies.

In Stacks, *m* was kept to 3 (the default value); *M* was incremented from 1 to 10 in two cases (specimens separated by 0 and 12 mutations at *mtMutS*) and from 1 to 7 in all other cases. Similarly, *n* was incremented from 1 to 10 (0 and 12 mutations cases) and from 1 to 8 (all cases). All combinations of *M* and *n* were not tested: only similar values of *M* and *n* were used together (two settings were used: *M* = *n* and *M* + 1 = *n*), as to (1) keep maximum levels of intra- and interindividual divergence levels close and (2) keep the number of Stacks analyses to a reasonable number. A total of 408 Stacks catalog construction tests were therefore performed using the denovo_map.pl script available in Stacks. Catalogs were parsed with the populations.pl script, where each sample was considered as a separate population, no missing data were allowed and a minimum of 10 reads per single-nucleotide polymorphism (SNP) was set.

In PyRAD v. 2.0, combinations of two values for Mindepth (3 and 6) and 3 values for Wclust (0.89, 0.93 and 0.96) were tested, resulting in 156 analyses. For these analyses, the maximum number of sites per read with a quality < 20 (NQual) was set to 4, the minimum number of samples in a final locus (MinCov) was set to 1 and the maximum proportion of shared polymorphic sites in a locus (MaxSH) was set to 10%. For this last parameter, which aims at detecting paralogs, preliminary tests showed that in our case, changing this value did not drastically affect the number of loci and SNPs detected. Finally, optional parameters were kept to default values.

Comparison of Stacks and PyRAD

To evaluate what proportion of loci was detected by both PyRAD and Stacks, a custom BLASTN search was performed (BLAST toolkit v. 2.2.25; Zhang *et al.*, 2000). Local BLAST databases were constructed using PyRAD sequences (locus file containing consensus sequences for each individual; PyRAD parameters *m* = 6 and Wclust = 93 and 89%) for three groups of specimens with different numbers of reads (Table 2). Stacks loci for these specimens (based on the locus file produced by the populations script, for which a single allele was retained per locus; denovo_map parameters *m* = 3, *M* = 4, *n* = 4, and *m* = 3, *M* = 10, *n* = 12) were then compared with the PyRAD database using BLASTN (percent identity set to 93 and 89%, word size 80 and 84 nt, ungapped alignments). The XML output of BLASTN searches was then parsed in bash using grep.

Table 2 Results of the BLASTN alignments performed between Stacks and PyRAD sequences

Specimen	Haplotype	Read category	No. of reads (M)	89% Divergence			93% Divergence		
				No. of loci (PyRAD)	No. of loci (Stacks)	Intersect (%)	No. of loci (PyRAD)	No. of loci (Stacks)	Intersect (%)
TER2044	11	High	5.82	6580	866	7.84	6720	607	5.54
JAC1018	J	High	5.49	3305	1851	24.57	2717	1202	21.46
TER7092	7	High	4.04	6867	1363	13.03	6862	1246	11.40
TER130424	9	Median	1.61	6151	1198	12.73	6323	850	8.86
TER13064	8	Median	1.61	6876	4183	39.89	6584	4607	42.72
TER13087	9	Median	1.60	5959	1131	13.81	6189	821	9.26
TER11101	4	Low	0.09	1046	228	1.15	944	138	0.64
TER13047	9	Low	0.08	1145	396	9.96	1107	297	8.67
TER11108	4	Low	0.04	441	50	2.49	384	32	1.04

The number of loci detected within nine individuals (with high-, medium- and low-read numbers) is presented for the two analyses performed on the entire set of 91 specimens. The number of quality-filtered reads is given in million.

Phylogenetic reconstruction and species delimitation

RAxML v. 8.0.9 (Stamatakis, 2006; Stamatakis *et al.*, 2008) was used on the CIPRES Portal (Miller *et al.*, 2010) to infer phylogenetic relationships among *Chrysogorgia* colonies, based on mitochondrial and nuclear sequences, using the GTRCAT1 model and automating boot-stopping. The mitochondrial phylogeny was inferred from the first 700 nt of the *mtMutS* gene (see above); the nuclear phylogeny was inferred using concatenated RAD loci obtained based on two parameter sets in Stacks, and one parameter set in PyRAD. The first Stacks set ('m3M4n4', *denovo_map* parameters $m=3$, $M=4$, $n=4$; *populations* script parameters $m=6$, $P=2$, $r=0.5$) corresponds to parameters that maximize the total number of loci detected while minimizing the divergence parameters (see 'Exploration of the divergence parameter space' section above). For this analysis, each *mtMutS* haplotype was considered as a separate population. The Stacks *populations* script parameters that were used signify that 50% missing data were allowed within each population, a locus had to be present in at least two populations to be included in the output and a minimum of six reads per SNP was required. The second Stacks set ('m3M10n12', Stacks script *denovo_map* parameters $m=3$, $M=10$, $n=12$; *populations* script parameters $m=6$, $P=2$, $r=0.5$) allowed more divergence between loci. The PyRAD data set ('m6s93') was constructed with $m=6$ and $W_{\text{clust}}=93\%$ (details above). In all analyses, the Atlantic colony JAC1018 was used as the outgroup.

Once clades were delimited with RAxML, a Discriminant Analysis on Principal Components (DAPC, Jombart *et al.*, 2010) was used to explore genetic structure within three clades represented by 18–31 colonies (see below). This method takes into account the multilocus genotype of each individual and forms clusters based on genetic similarity without considering a model of evolution. We also used TESS (Durand *et al.*, 2009) to investigate population structure using the conditional auto-correlative admixture model with a spatially explicit, Bayesian framework. In TESS, the deviance information criterion was used to compare population structure in the presence of different numbers of clusters (the maximum number of cluster K was set to the total number of individual in the tested clade; for example, K was set from 2 to 18 for clade 1). Five replicate runs were used per K , with 1200 MCMC steps and a 200-step burn-in. The best K was determined by minimizing deviance information criterion and its variance; once the best K was determined, a longer analysis with 12 000 steps and a 2000-step burn-in was run to obtain reliable individual assignments. The *populations* script in Stacks was rerun to keep only one SNP per locus, to minimize the probability of coanalyzing linked markers. The Stacks *m3M4n4* data set was chosen for these analyses for two reasons: (1) the DAPC and TESS analyses are run within clades at shallow phylogenetic depths and (2) as only one SNP/locus is retained, divergence level should be kept minimal to prevent the inclusion of non-homologous loci. The DAPC analysis was run using adegenet in R (Jombart, 2008; R Development Core Team, 2014).

RESULTS

Mitochondrial typing and RAD-tag sequencing

A total of 12 *mtMutS* haplotypes were detected among the 91 colonies investigated, 10 of which were from NC, 3 from PNG, 1 from the northwestern Atlantic and 2 being shared between NC and PNG. The biogeography of these mitochondrial haplotypes at these locations is further discussed in Pante *et al.* (2012a,b). A total of 236 million raw reads, corresponding to 35 463 Mbp were produced on two HiSeq2000 lanes. The number of quality-filtered reads (in millions) per colony varied between 0.04 (TER11108) and 5.82 (TER2044), with a median of 1.6. There was a significant correlation between the number of quality-filtered reads per colony and haplotypes (Kruskal–Wallis X^2 -test = 25.11, $df=13$, $P=0.02$), haplotypes 6 and 10, for instance, yielded fewer reads than other haplotypes (haplotype-10 colonies were sampled from depths down to 880 m, and haplotype-6 colonies had remarkably small polyps that may have been particularly sensitive to prolonged times to preservation).

Loci, SNPs and indel cataloging using Stacks and PyRAD

Results from both pipelines (Stacks and PyRAD) show variations in the number of loci and SNPs depending on the set of parameters used (Figures 1a–e and 1g–k), as well as the mitochondrial genetic distance between samples (Figure 1f). For Stacks, as the mitochondrial genetic distance among included samples decreases, both the total number of loci and the number of polymorphic loci increases (Figures 1a and b). The former ranges from a few loci to more than 2000, whereas the latter ranges from a few loci to ~1000, depending on the set of parameters used. When related to the time of divergence (in Myr, based on mtDNA), the total number of loci obtained decreases exponentially (Figure 1f). Inversely, the percentage of polymorphic loci is lower for more closely related colonies (~40%) than for distantly related colonies (~90%; Figure 1c). These three measures (number of loci, number of polymorphic loci and percentage of polymorphic loci) show the same response to an increase in divergence parameters M and n , namely a rapid increase followed by a plateau. This plateau is reached for the *m3M4n4* set of parameters. Conversely, the number of SNPs increases drastically without reaching a plateau, from a few SNPs for the most stringent set of parameters and the most distantly related colonies to around 3000 for the most closely related colonies and the most relaxed set of parameters (Figure 1d). Thus, the effect of increasing mitochondrial genetic distance among samples or decreasing stringency of parameters is to increase SNPs densities, from one SNP every 250 bp to one SNP every 20 bp (Figure 1e).

Results of the PyRAD analyses follow the general trends observed for the Stacks pipeline. These trends are an increase in total number of loci and polymorphic loci (Figures 1g and h) for more relaxed parameters sets, as well as for more closely related colonies. As for Stacks, more distantly related specimen pairs have fewer loci than for closely related ones, but a larger proportion of those is polymorphic (Figure 1i). Although the percentage of polymorphic loci shows similar ranges of values for Stacks and PyRAD, the total number of loci as well as the number of polymorphic loci are almost doubled (from 2000 to almost 4000 and from 1000 to almost 2000, respectively). The same pattern is observed for the number of SNPs and SNP densities (Figures 1j and k): PyRAD output differs from Stacks output by a factor of almost two, resulting in SNPs densities twice as high (from one SNP every 130 bp to one SNP every 20 bp). Finally, unlike Stacks, PyRAD allows for indels within loci. The percentage of loci containing indels increases with less stringent sets of parameters (Figure 1l). Depending on the pair of samples considered, this measure varies from a few percent to almost 40%. For PyRAD, the number of cataloged loci decreased rapidly with the number of specimens included in the analysis (with significant drops corresponding to the number of individuals in the haplotype clades revealed by the phylogenetic reconstruction, see below; Figure 2). Most loci bore less than three SNPs even when 10 polymorphisms were allowed on a single RAD locus (Figure 2).

We measured the proportion of loci cataloged by Stacks that was also detected by PyRAD using custom BLASTN database searches. Overall, 0.6–42.7% of loci detected by Stacks were present in the PyRAD catalog. This pattern is partly explained by the proportion of PyRAD loci with indels (see above), but might also be influenced by the differential detection of repeated regions (that is, deleveraging algorithm in Stacks), or the number of reads per individual (the proportion of loci in common between Stacks and PyRAD was lower for individuals with fewer reads; Table 2).

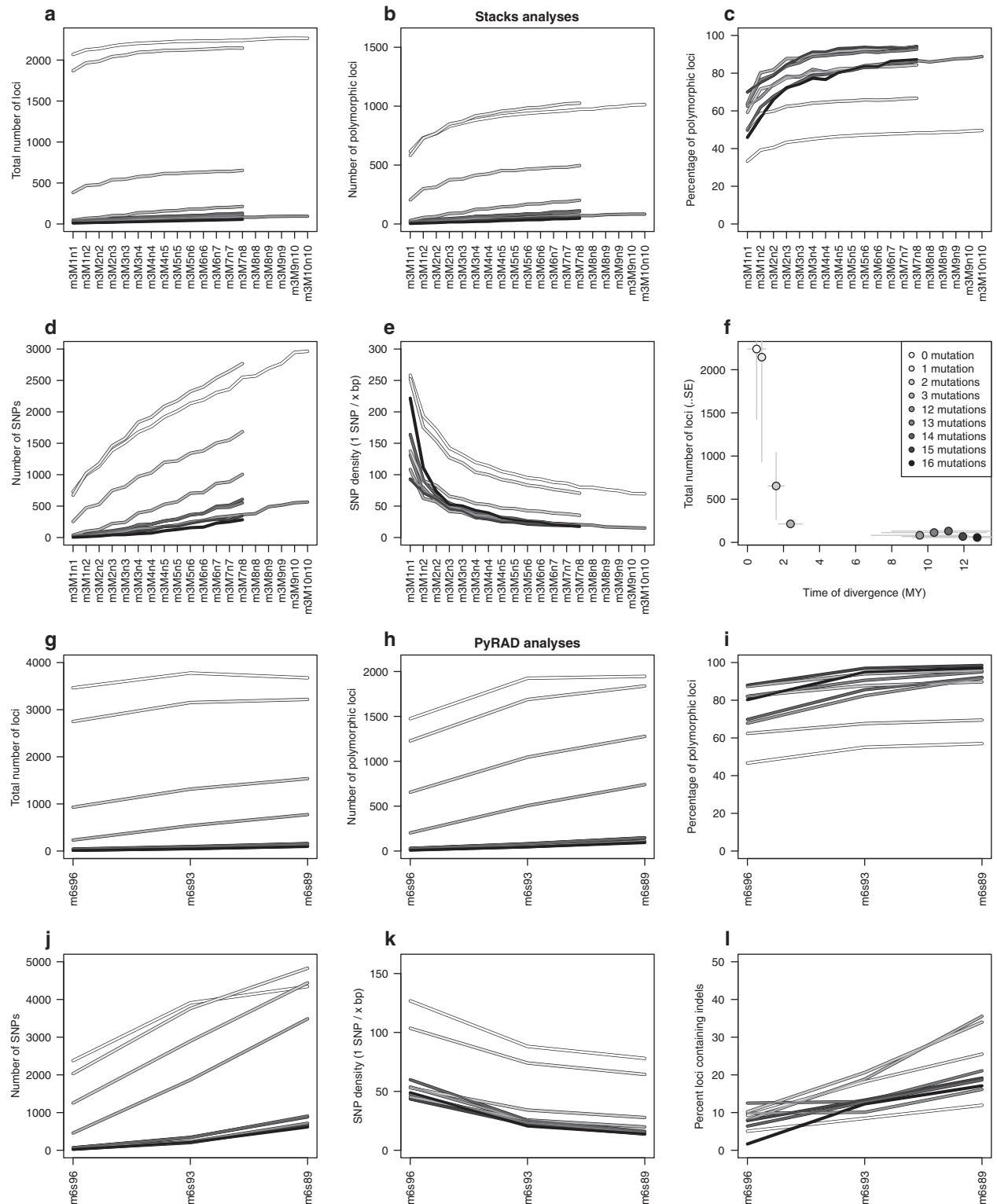


Figure 1 Comparison of locus detection for Stacks (a–f) and PyRAD (g–l). The number of loci, SNPs and indels detected for specimens separated by 0–16 mutations at the mitochondrial *mtMutS* gene are shown for the different read coverage (*m* parameter) and divergence levels (*M* and *n* parameters, see text). In PyRAD analyses, ‘s’ corresponds to the ‘Wclust’ parameter. A full color version of this figure is available at the *Heredity* journal online.

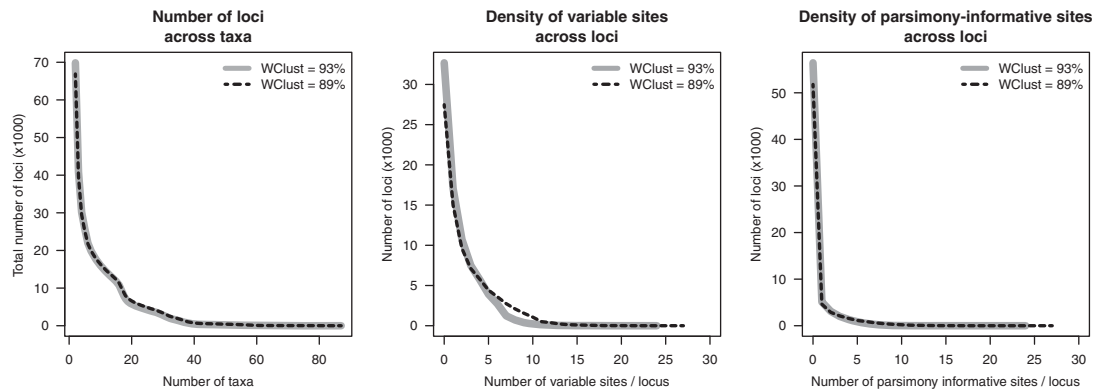


Figure 2 Information content of the locus catalog built by PyRAD for all 91 *Chrysogorgia* specimens. WcLust: percent divergence permitted between loci within and across specimens; in addition to the 93% WcLust level used to infer the *Chrysogorgia* phylogeny, the 89% WcLust level was tested here. A full color version of this figure is available at the *Heredity* journal online.

Phylogenetic reconstruction and species delimitation

The automatic boot-stopping method implemented in RAxML yielded 1000 bootstrap replicates for the mitochondrial phylogeny (91 taxa \times 700 nt), 500 replicates for the Stacks RAD phylogenies (91 taxa \times 1 080 352 nt, 11 872 loci for the first data set and 1 146 054 nt, 12 594 loci for the second data set), and 200 replicates for the PyRAD phylogeny (91 taxa \times 6 120 523 nt, 69 851 loci). The proportion of gaps and undetermined characters ranged between 83 and 84% for Stacks and was 92% for PyRAD. The three RAD phylogenies were similar but not identical, the second Stacks data set being better resolved than the first, and the PyRAD data set being better resolved than the Stacks sets (nodes with bootstrap $> 70\%$: 19% for m3M4n4, 29% for m3M10n12, 40% for m6s93; Figure 3). Divergence levels were much higher in the RAD phylogenies compared with the mitochondrial phylogeny. For instance, the groups composed of haplotypes 9 and 10 were separated by a distance of 0.001 substitution/site on the *mtMutS* tree, whereas these clades were separated by 0.27 and 0.25 substitutions/sites on the m3M4n4 and m3M10n12 RAD phylogenies, respectively (Figure 3).

Out of nine mitochondrial haplotypes represented by more than one individual, six formed well-supported monophyletic groups on the RAD phylogenies, for all data sets. One of these clades (corresponding to haplotype 10) contained specimens from both NC and PNG. The group formed by mitochondrial haplotype 7 was polyphyletic on the RAD phylogenies, with specimens grouping in two well-supported clades on the PyRAD phylogeny: one composed of five closely related NC specimens and one composed of three more divergent PNG colonies (this clade was split in two on the Stacks phylogenies). Specimens characterized by *mtMutS* haplotype 7 may therefore belong to at least three distinct species. On the other hand, specimens characterized by three distinct mitochondrial haplotypes (2, 8, 13) clustered into a single, well-supported clade (with the exception of one individual, TER13034, haplotype 8, which clusters well outside this clade). These three haplotypes, which form a paraphyletic group on the mitochondrial phylogeny and are one to two mutations different from each other, would therefore be considered as one evolutionary unit based on the RAD phylogenies (and population clustering analyses with DAPC and TESS failed to detect structure within this clade; see below). Finally, out of three singleton haplotypes (J, 13, 14), two (J, 14) sit on long branches and are clearly differentiated from other haplotypes using RAD-tag data.

We ran a DAPC on the three clades that contained the most colonies (clade 1: 18 colonies of haplotype 9; clade 2: 20 colonies of

haplotype 4; clade 3: 31 colonies of haplotypes 2, 8, 13). Within these clades, 3685, 1470 and 8201 loci were retained (with 25, 42 and 55% missing data, respectively). In all three cases, DAPC failed to detect intraclade genetic structure, as the most likely number of group (based on BIC, discounting the scenario in which each sample belongs to its own group), in each case, was one (Supplementary Figure S1). The spatially explicit admixture model implemented in TESS also failed to detect genetic structure within clades 1 and 3, but suggested the presence of three clusters in clade 2, these clusters being composed of colonies sampled (1) on the slope of New Caledonia, (2) Munida Seamount (Norfolk Ridge) and (3) Jumeaux Ouest Seamount (Norfolk Ridge; Supplementary Figure S1). The population genetics of *Chrysogorgia* will be further discussed in a separate study.

Detection of environmental contaminants

As octocoral DNA was extracted from whole polyps rather than dissected, internal tissue, some loci may come from environmental contaminants such as bacteria. To evaluate the prevalence of such loci, we blasted all the loci that were cataloged for the m3M4n4 Stacks data set from individual JAC1018 ($n = 1202$). The BLASTN algorithm (Altschul *et al.*, 1997) was used to match RAD loci to the non-redundant NCBI nucleotide database, using 10^{-3} as a statistical significance threshold (e-value). Most sequences (92.6%) could not be assigned to a match in the nucleotide database and 4.5% of loci were similar to bacterial sequences (78–100% similarity between match and query). A single locus matched human mitochondrial DNA (84% similarity); other matches ($n = 34$) included other invertebrates and plant sequences. Given (1) the small prevalence of potential contaminants, (2) our inability to determine whether these loci really belong to contaminant DNA or correspond to coral sequences which closest matches are non-cnidarian taxa and (3) the large number of Stacks analyses performed (> 400), we decided to run our analyses without trying to filter loci from exogenous DNA sources.

DISCUSSION

A critical decision in RAD analyses is the way the sequencing data are filtered to get to the final SNP data set. This process goes through several steps to ensure that the final loci will correspond to homologous sequences. The main filters involve several quality filters (sequencing quality, sequencing depth) as well as several similarity thresholds aimed at identifying the different allelic states of homologous loci. Finally, for each sample, an algorithm is used to tell apart sequencing errors from real mutations to conduct the final SNP

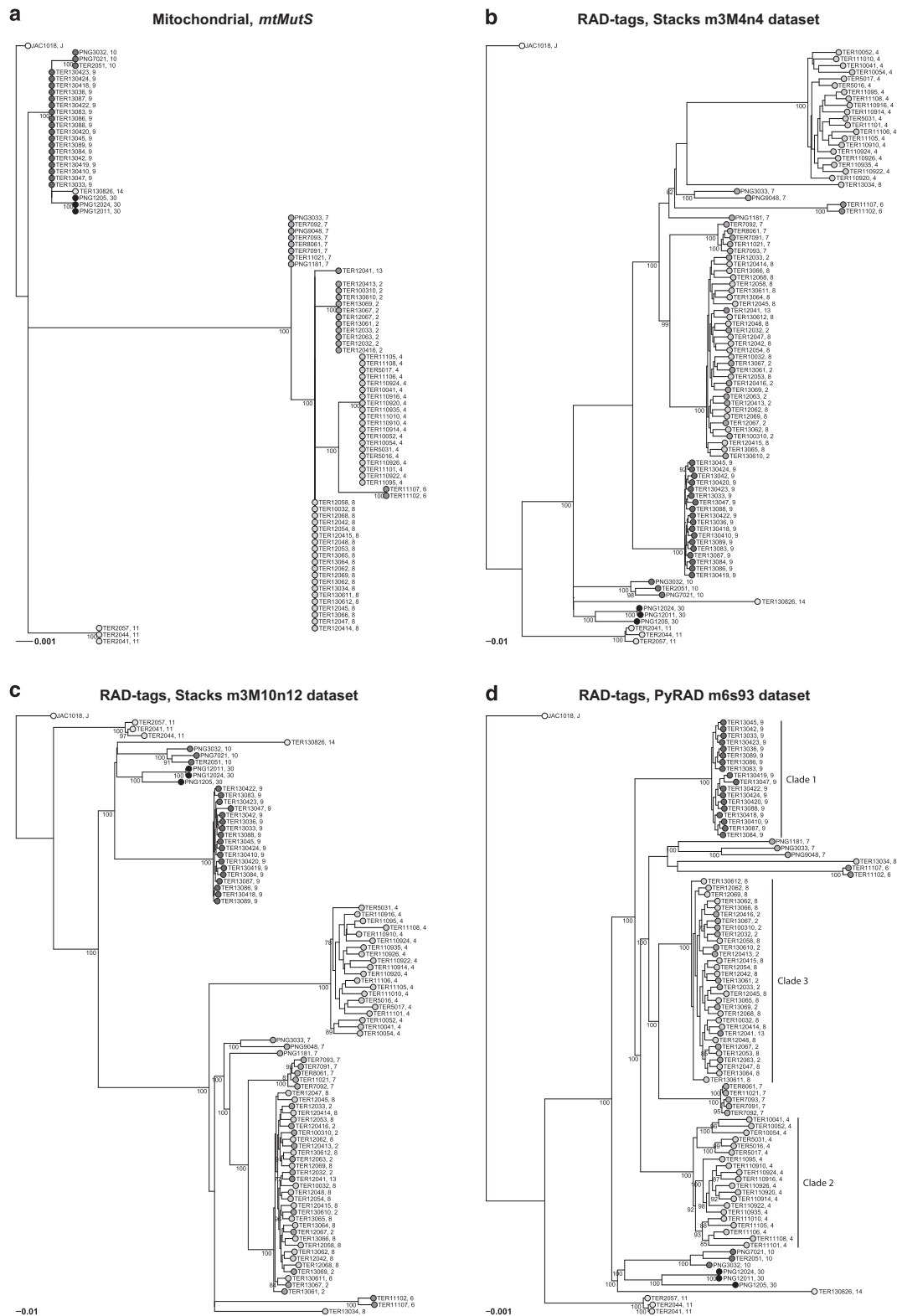


Figure 3 Maximum likelihood phylogenetic trees inferred using RAxML for the mitochondrial *mtMutS* data (a) and RAD loci (b–d). Bootstrap node support (1000 replicates for a, 500 replicates for b, c and 200 for d) is presented only for nodes with $\geq 70\%$ support. At the tips, colored dots, which represent *mtMutS* haplotype membership (each color represents a unique haplotype), are followed by specimen identifiers and haplotype numbers. Each tree was rooted to the Atlantic specimen (JAC1018, haplotype J). Genetic structure within clades 1, 2 and 3 were further investigated using a DAPC and TESS (see text and Supplementary Figure S1). Scale bars: substitution/site. A full color version of this figure is available at the *Hypertension Research* journal online.

calling. Even though the overall process is quite similar for Stacks and PyRAD analyses pipelines, a strict comparison of their results is not straightforward as they use sets of parameters that differ to some extent. A main difference between these two pipelines is in the assessment of similarity of loci: Stacks uses a strict similarity criterion (maximum number of mutations) to cluster reads into loci, whereas PyRAD uses an overall similarity criterion, after an alignment step, allowing for the presence of indels within clusters. This should be a critical difference when comparing genetically more-distant samples as indels are more likely to occur, and would thus result in sequences being assigned to different loci using Stacks (which will then be excluded from the final catalog as not present in all individuals), whereas PyRAD would theoretically allow these reads to be considered as homologous loci.

Our results show that more loci are recovered using the PyRAD pipeline. Despite these differences, general trends are similar using both pipelines. First, fewer loci and SNPs are recovered when comparing more genetically distant samples. This result is expected and has been anticipated through simulation (Cariou *et al.*, 2013) and observed empirically (Cruaud *et al.*, 2014). Our data show an exponential decay of the number of loci recovered as a function of divergence time of samples. Second, the stringency of the filtering process has a significant effect on the number of loci and SNPs identified. Indeed, higher minimum depth of sequencing thresholds and higher similarity threshold lead to fewer loci being identified. This trend is observed regardless of the level of genetic divergence between samples, but it seems to be accentuated when samples are more closely related.

Despite the similarities in general trends, quantitative and qualitative differences are observed in the outputs of each pipeline. Indeed, whatever the set of parameters used, almost twice as many loci are identified using PyRAD compared with Stacks. This difference cannot be solely attributed to the management of indels as our results show that the percentage of loci containing indels is usually around 5–20% and never reaches 40% whatever be the genetic distance between samples and the parameters set. Another interesting result is that PyRAD is not simply adding extra loci to the total loci identified by Stacks: only half of the loci identified using Stacks are also present in the PyRAD loci catalogs. It is thus necessary to invoke other filtering processes and differences in algorithm to explain these differences in output. More thorough analyses would be needed to identify precisely what are the main sources of divergence in the processing of raw data, in addition to the treatment of indels.

One major result is the remarkable loss of homologous loci with increasing divergence among specimens with different mitochondrial haplotypes. For instance, compared with specimens sharing the same haplotype, specimens two mutations apart at *mtMutS* (estimated divergence of 1–2 Myr) had on average 70% fewer homologous loci (Stacks analysis at *m3M7n8*). Within the genus, specimens from mitochondrial clades 16 mutations apart (that is, the highest divergence level included in our study, estimated between 9 and 16 Myr) share 97% fewer loci. This rate of loss of homologous RAD tags is far greater than what has been observed in cetaceans (Viricel *et al.*, 2014), for which 66% of homologous loci were retained at the interfamilial level (short-beaked common dolphins, *Delphinus delphis*, vs harbor porpoise, *Phocoena phocoena*; estimated divergence of 14–19 Myr) compared with the intraspecific level (within *Delphinus delphis*). Comparisons within cetaceans were performed using the same custom pipeline as used in the present study, using Stacks parameters *m3M3n3* (the results for corals were similar when comparing *m3m3n3* to *m3M7n8*).

The differences observed between our study and that of Viricel *et al.* (2014) may be explained by various factors. For example, the choice of restriction enzyme was different (*SbfI* here, *NotI* for Viricel *et al.*), and differences in genome composition (most importantly GC content and size) are unknown. Although both studies were conducted with two lanes of Illumina HiSeq2000 sequencing (conducted by Eurofins Genomics in both cases), throughput may have been influenced by the quality of genomic DNA (trawled deep-sea samples here, stranded animals for Viricel *et al.*). These various factors may have significantly influenced the number of cut sites. Our comparisons might also be significantly affected by the precision of the molecular clocks available. Divergence times between cetacean families were inferred based on fossil evidence (see references in Viricel *et al.*, 2014), whereas no such fossil-calibrated molecular clock exists, to the best of our knowledge, for octocorals. The *mtMutS* divergence rates estimated by Lepard (2003) are based on a group of shallow-water octocorals that may evolve faster than the deep-sea *Chrysogorgia* (a long standing question in deep-sea biology is whether evolutionary process take longer in deeper water, compared with shallower waters; for example, Wilson and Hessler, 1987), and rely on a geological event (rising of the Isthmus of Panama), which can introduce further bias.

The exploration of divergence parameter space, as outlined above, was made using pairs of specimens, and not allowing any missing data. Stacks and PyRAD can build catalogs with loci shared by a set proportion of individuals within predefined groups. Hence, our phylogenetic matrix based on over 12K loci (Stacks parameters *m3M10n12*) resolved most deeper nodes of the tree despite 83–84% of missing data. Similarly, Cruaud *et al.* (2014) constructed a phylogeny of 18 species of the beetle genus *Carabus*, and found that the deepest node of the tree (17 Myr divergence between species) was characterized by 67% of missing data but strong statistical support. Jones *et al.* (2013) reconstructed phylogenetic relationships among congeneric species of swordtail and platyfish (*Xiphophorus* sp.) that diverged <3 Myr, and estimated up to 70% missing data (ingroup data). They noted, however, that missing data had little effect on tree topology and branch support. The rate of loss of homologous loci observed in swordtail and platyfish is more on par with what we observed for *Chrysogorgia* than what was reported for cetaceans and *Carabus* beetles, and further emphasizes that (1) the utility of RAD sequencing for phylogenetic reconstruction may be taxon dependent and (2) molecular clocks must be critically interpreted. It must be underlined, however, that notable differences in tree topologies were observed between the three inferred RAD phylogenies, such as deep but well-supported nodes (for example, relative positions of clade 3 and haplotypes 6, 7 and 8).

RAD-tag sequencing has also proven very useful in testing the criterion used for our primary species delimitation hypotheses, namely that single mitochondrial *mtMutS* haplotypes discriminate species that fit within the General Lineage Concept of species as defined by de Queiroz (1998). Indeed, a large numbers of variable loci could be cataloged within and among closely related colonies (sharing the same *mtMutS* haplotype, and therefore putatively belonging to the same species) and more distantly related colonies (separated by 1–16 mutations at *mtMutS*, putatively belonging to different species), allowing us (1) to plot our primary delimitation hypotheses onto well-supported phylogenies and (2) to explore the spatial structure of populations. Three patterns were evidenced from the data: (1) in the majority of cases, we noted a complete congruence between *mtMutS* haplotypes and RAD clades (6/9 non-singleton haplotypes and 2/3 singleton haplotypes); (2) in one case, incomplete congruence was noted (with PyRAD, haplotype 7 corresponding to two RAD clades

(one NC, one PNG) that did not form a monophyletic group; (3) in one case, a single RAD clade included specimens with different (but closely-related) haplotypes. This result is significant for octocoral taxonomy and systematics, as *mtMutS* has been widely used to assist species delimitation across a large number of families (for example, review of McFadden *et al.*, 2010). Although morphological, mitochondrial (Pante and Watling, 2012) and genomic data (this study) all point to the utility of *mtMutS* for delimiting *Chrysogorgia* species, its resolution should be interpreted in two ways. First, as we did not find 100% congruence between RAD clades and *mtMutS* haplotypes, and tested only a restricted set of putative species, *mtMutS* should still be considered as one of the first steps in an integrative taxonomic loop incorporating more variable markers (for example, Schlick-Steiner *et al.*, 2010; Kekkonen and Hebert, 2014). Second, the evolutionary speed of *mtMutS* may well vary among octocorals, and its resolving power may therefore vary from one group to another (for example, Baco and Cairns, 2012). Nevertheless, combining mitochondrial markers such as *mtMutS* and RAD-tag data will without doubt be of tremendous value for testing the large number of outdated species hypotheses within the Octocorallia.

DATA ARCHIVING

Mitochondrial haplotypes were deposited on GenBank (Supplementary Table S1). Phylogenetic data were deposited on Dryad: doi:10.5061/dryad.rp8d0.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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An inter-ocean comparison of coral endemism on seamounts: the case of *Chrysogorgia*

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ABSTRACT

Aim The biogeography of seamount fauna remains poorly known, with less than 1% of the world's seamounts having been investigated. Here, we report data on the geographical isolation of species in the octocoral genus *Chrysogorgia* from south-west Pacific seamounts and slopes, and contrast the results with patterns observed in the north-western Atlantic.

Location Seamounts of the Norfolk Ridge (NR) and Loyalty Ridge (LR), the slope of New Caledonia, and the Matthew and Hunter Islands, south-west Pacific Ocean, with comparative material from the Pacific and Atlantic oceans.

Methods The mitochondrial gene *mtMutS* was used to measure diversity within *Chrysogorgia*. Community structure was analysed using rarefaction, multivariate analyses, parsimony analysis of endemism and analysis of molecular variance. The impact of underestimating species richness when using mitochondrial haplotypes was tested using simulations.

Results Six hundred and thirty-four colonies and 31 haplotypes were sampled from New Caledonia. Contrary to what was observed in the north-western Atlantic, seamount-scale endemism of south-west Pacific *Chrysogorgia* was substantial (23% and 39% for haplotypes with $n \geq 20$ and $n \geq 2$, respectively). LR sheltered 64% of the New Caledonian haplotype diversity. Assemblages were structured less by habitat type (slope versus seamounts) than by depth. Rarefaction analyses suggested that LR and NR seamounts hold more species than the New Caledonian slope, but additional sampling in the south-western Pacific (133 colonies) revealed that some seemingly geographically restricted haplotypes from New Caledonia have wide geographical distributions, reaching as far as Taiwan.

Main conclusions The distribution of Pacific *Chrysogorgia* is characterized by high levels of rarity, patchiness and diversity, with the levels of seamount-scale and seamount-chain-scale endemism higher than in the Atlantic. We hypothesize that the contrast between the wide geographical distribution of Atlantic *Chrysogorgia* haplotypes and the higher proportion of endemics in the Pacific is largely explained by differences in depth between the seamounts of these two regions.

Keywords

Atlantic Ocean, marine biogeography, marine connectivity, Octocorallia, Pacific Ocean, parsimony analysis of endemism, seamount, species delimitation.

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INTRODUCTION

Seamounts, typically defined as undersea mountains that rise above the sea floor, are among the most ubiquitous underwater features in the oceans (Wessel *et al.*, 2010), and constitute one of the largest marine biomes, with a total

surface area similar to that of Europe and Russia combined (Etnoyer *et al.*, 2010; Yesson *et al.*, 2011). The geological and hydrographical setting in which seamounts are found makes them a noteworthy system for the study of deep-sea biogeography. Most seamounts are volcanic in origin, and they are commonly characterized by hard substrates and relatively

steep slopes, features that are rare in the deep sea (Rogers, 1994). They emerge at island arcs, mid-ocean ridges and in intraplate hotspot settings, forming chains (Wessel, 2007). Because of these geological attributes, seamounts may act as stepping stones between patches of suitable habitats on continental shelves and oceanic ridges, and contribute to pan-oceanic dispersal (Ekman, 1953; Hubbs, 1959). On the other hand, hydrographical features such as Taylor caps, local upwelling, jets and eddies (reviewed in Rogers, 1994), and sheer geographical isolation, may significantly impede dispersal between habitat patches (Parker & Tunnicliffe, 1994).

One question that has generated great interest and debate among deep-sea biologists is whether the fauna associated with seamounts is highly endemic. The topographical and hydrographical conditions associated with seamounts could contribute to faunal isolation and the accumulation of highly endemic taxa (Hubbs, 1959). Wilson & Kaufmann (1987) provided the first global assessment of levels of endemism on seamounts, and reported that 11.6% of fishes and 15.4% of invertebrates were endemic to individual seamounts or seamount groups. Since this seminal review, many estimates of endemism have been published, ranging from 0% to 100%, reflecting various geographical ranges and sampling efforts (Stocks & Hart, 2007). Most notably, Parin *et al.* (1997) compiled information from 22 seamounts of the Nazca and Sala y Gómez chains in the south-eastern Pacific, and found that 44–51% of fishes and invertebrates were seamount endemics. Richer de Forges *et al.* (2000), summarizing the results of 24 exploration cruises, found that 29–34% of 850 fish and invertebrate species collected from the Norfolk Ridge, Lord Howe and Tasmanian seamounts were potential endemics.

From these studies emerged the general paradigm that seamounts harbour high levels of endemism (Rowden *et al.*, 2010a). Even though this paradigm is largely unsupported by recent studies (Samadi *et al.*, 2006; Hall-Spencer *et al.*, 2007; O'Hara, 2007; Stocks & Hart, 2007; Lundsten *et al.*, 2009a,b; McClain *et al.*, 2009; Howell *et al.*, 2010), the debate is not closed. Indeed, a minuscule proportion of seamounts have been biologically sampled (< 1%; Staudigel *et al.*, 2010), and the scarcity of data prevents us from conceptualizing the processes that shape and maintain seamount faunal assemblages (see, for example, reviews by McClain, 2007; Clark *et al.*, 2012). In addition, most current estimates of endemism are based on morphological variation, which can be a highly biased metric of biodiversity. Molecular studies are also subject to biases, in that it can be difficult to establish whether a lack of genetic divergence reflects past or current faunal connectivity (Clark *et al.*, 2010; Miller *et al.*, 2010). It is therefore important to integrate taxonomic and biogeographical approaches when considering questions of faunal isolation (Castelin *et al.*, 2010). Characterizing these faunal assemblages is becoming increasingly important for conservation, as seamounts can be targets of commercial mining and fishing (e.g. Clark *et al.*, 2010; Williams *et al.*, 2010; Schlacher *et al.*, 2014).

Chrysogorgia Duchassaing & Michelotti, 1864 is a relatively diverse genus of Octocorallia (Cnidaria, Anthozoa). It is

globally distributed, and is found both on slopes and on seamounts, where it can be locally very abundant (Watling *et al.*, 2011). The bathymetric range of *Chrysogorgia* is extremely large (Pante *et al.*, 2012a, and references therein), and encompasses the entire depth range of catalogued seamount summits and slopes (Stocks, 2010). In addition, the genus appears to be monophyletic based on current data (Pante *et al.*, 2012a), offering opportunities for specific hypotheses about the historical biogeography of species and species groups to be tested. Finally, the relative congruence between morphotypes and mitochondrial *mtMutS* haplotypes described in four North Atlantic species suggests that endemism can be investigated reliably at the species level (Pante & Watling, 2012). This result was recently confirmed by comparing genome-scale data to *mtMutS* haplotypes (Pante *et al.*, 2014).

Based on these characteristics, *Chrysogorgia* offers a model system that is well suited to estimating species endemism at different spatial scales, across the spectrum of seamount environments (e.g. depth range or geological origin) and geographical locations (e.g. latitude or isolation from continental margins) in a phylogenetically comprehensive manner. Here, we investigate the geographical distribution of *Chrysogorgia* species from seamount and non-seamount environments in the south-western Pacific, and contrast the patterns of species distribution with those observed in the north-western Atlantic (Thoma *et al.*, 2009). To the best of our knowledge, this is the first study to look at the seamount-scale endemism of a genus in different ocean basins.

MATERIALS AND METHODS

Sampling and genotyping

Specimens from the New Caledonian exclusive economic zone were collected during the Muséum national d'Histoire naturelle (MNHN)/Institut de Recherche pour le Développement (IRD) *Terrasses* and *ExBoDi* cruises of 2008 and 2011 on the NR, LR, eastern slope of New Caledonia, and Matthew and Hunter Islands (MH) (Figs 1 & 2, and see Appendices S1 & S2 in Supporting Information). The *Terrasses* and *ExBoDi* cruises are part of a long-term research endeavour – the Tropical Deep-Sea Benthos (TDSB) program (Bouchet *et al.*, 2008; details of the cruises are available at <http://expeditions.mnhn.fr/program/tropicaldeep-seabenthos>) – and relied on the combined use of a beam trawl and a dredge to maximize the types of substrates and faunal diversity collected. During the *Terrasses* cruise, 256 *Chrysogorgia* colonies were collected, 213 of which were successfully genotyped (see below). An additional 439 and 421 colonies were collected and genotyped, respectively, during the *ExBoDi* cruise. Specimens from other cruises in the Pacific (including the TDSB cruises *BIOPAPUA*, *EBISCO*, *MADEEP*, *Norfolk 2*, *PAPUA NIUGINI*, *Salomon 1*, *Salomon 2*, *SMIB 4* and *TAIWAN2013*) provided additional specimens for biogeographical comparisons ($n = 133$; Appendix S1). For comparison, the study of Thoma

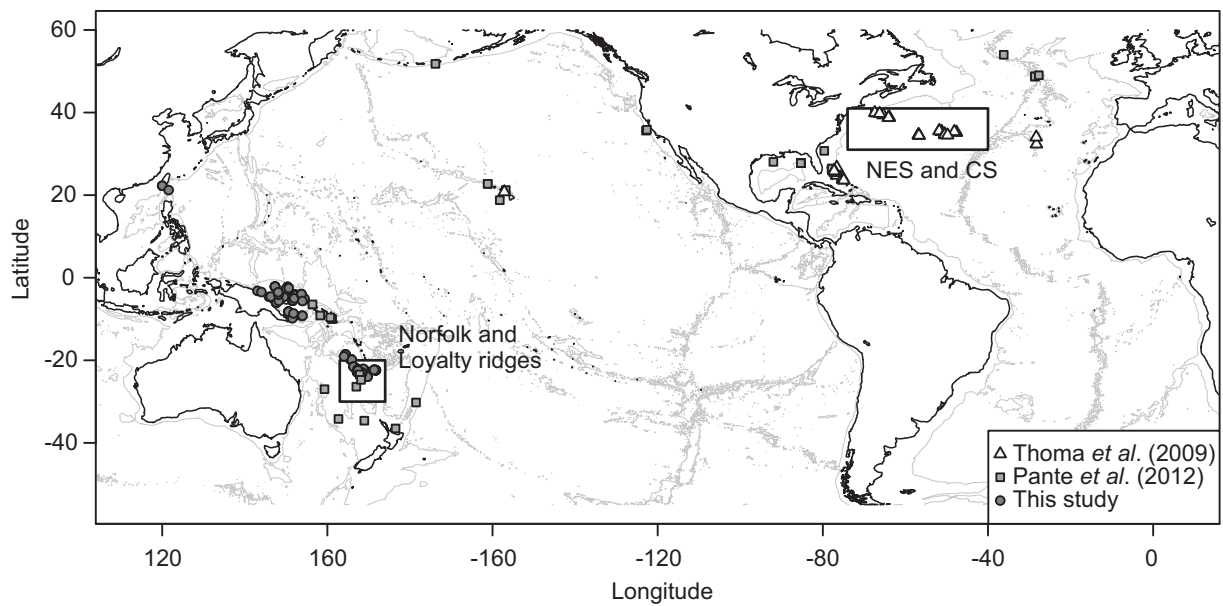


Figure 1 World map with the two major sampling regions and the sampling locations of the *Chrysogorgia* specimens used in biogeographical comparisons. Samples used in previous studies are plotted using different symbols. NES, New England Seamounts; CS, Corner Seamounts.

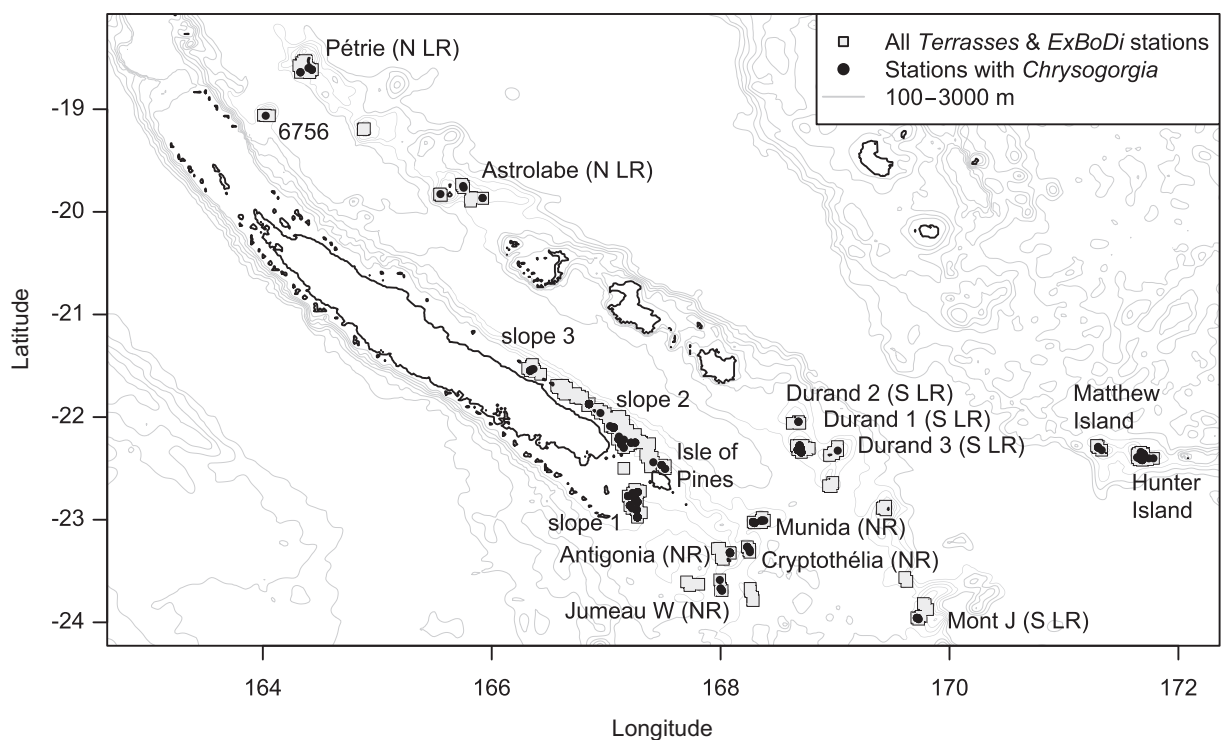


Figure 2 Bathymetric map of New Caledonia and parts of the Norfolk and Loyalty ridges. All stations sampled during the *Terrasses* and *ExBoDi* cruises are plotted as grey squares. Stations containing at least one *Chrysogorgia* colony are marked by black circles. Isobaths are plotted every 500 m from 100 to 3000 m. Slope stations were divided into three geographically discrete areas for biogeographical analyses. The feature labelled '6756' is a nameless seamount (Allain *et al.*, 2008) adjacent to the New Caledonian slope. Although a *Chrysogorgia* specimen was collected from this seamount, it could not be genotyped. LR, Loyalty Ridge; NR, Norfolk Ridge.

et al. (2009) in the north-western Atlantic included 24 *Chrysogorgia* colonies among 188 octocorals. Sampling maps (Figs 1 & 2) were made with MARMAP (Pante & Simon-Bouhet, 2013; R Core Team, 2014).

Specimens were preserved in 80–100% ethanol or RNAlater (Ambion, Austin, TX, USA), or frozen at -80°C . Protocols for DNA extraction, PCR and sequencing are detailed in Pante *et al.* (2012b). The 5' region of *mtMutS* was PCR-amplified using the primers ND4L2475F and MUT3458R. For specimens with sheared DNA template, internal primers were used (Pante *et al.*, 2012a). Haplotypes were defined as unique *mtMutS* sequences, as described in Thoma *et al.* (2009).

Evaluation of putative endemism, haplotype richness and sampling biases

Previous work in the north-western Atlantic that compared genetic variability with morphological data suggested that *mtMutS* haplotypes had the power to resolve species of *Chrysogorgia*, at least at a regional scale (Pante & Watling, 2012), although the number of haplotypes studied (four) was relatively small. It is therefore possible that, among our Pacific samples, a single haplotype may represent multiple species of *Chrysogorgia*, reducing our power to detect narrow geographical ranges (e.g. Baco & Cairns, 2012). To test whether unrecognized species diversity significantly affects our estimates of connectivity between island slopes and seamounts, we performed computer simulations in which haplotypes were split into two species of equivalent biogeography (Appendix S2).

To test the hypothesis that seamounts and island slopes harbour different species, we analysed the site \times haplotype matrix using non-metric multidimensional scaling (NMDS; Kruskal, 1964a,b) and analysis of similarities (ANOSIM; Clarke, 1993). Sites are groups of stations as presented in Fig. 2 and Appendix S1. We also grouped sites into five regions (Fig. 2) to perform hierarchical clustering. Jaccard's index was used to compute distance matrices from presence/absence data, because dredging and trawling are semiquantitative sampling methods that may not capture the true abundance of organisms. Analyses were performed in R using the package VEGAN 2.2-1 (Oksanen *et al.*, 2015).

To test the hypothesis that seamounts harbour more endemics than island slopes, we looked for species that were limited to individual seamounts and seamount chains. We completed this qualitative survey by parsimony analysis of endemism (PAE; Rosen & Smith, 1988). This method relies on parsimony to infer the relationship among sites, based on their shared haplotypes. Sampling sites were grouped by region (slope, NR, southern LR, northern LR and MH), for which species occurrence was coded as 0 (absent), 1 (present at one site) or 2 (present at more than one site). A 'zero vector' containing no species was included in the region \times haplotype data matrix to polarize characters (Morrone, 1994). The most parsimonious tree was searched using nearest-neighbour interchange rearrangements, and 1000 inde-

pendent replicates were run. The consistency index (CI) and retention index (RI) were calculated to evaluate how well the haplotype distribution data fitted the most parsimonious tree (i.e. the degree of homoplasy). PAE was performed in R using the package PHANGORN (Schliep, 2011).

To test the hypothesis that seamount communities are richer than island-slope communities, haplotype richness was estimated using sample-based rarefaction, because the total numbers of individuals and stations sampled varied among localities (Gotelli & Colwell, 2001). Estimated species richness (as computed in Colwell *et al.*, 2012) was scaled both using samples (Colwell *et al.*, 2012) and using individuals (Gotelli & Colwell, 2001), in order to ease interpretation. Computations were performed in ESTIMATES 9.0 (Colwell, 2013). No rarefaction analysis was performed on the north-western Atlantic data, because specimen collection during dives was non-random. Similarly, data from NR collected on TDSB cruises prior to the *Terrasses* cruise (Bouchet *et al.*, 2008) were not used in the rarefaction analysis, because their sampling of *Chrysogorgia* was more qualitative than quantitative.

Finally, we tested whether *mtMutS* haplotypes that share a distributional pattern (specialist on seamounts or island slopes, or generalist) are phylogenetically close. To do so, we inferred the phylogenetic relationships of all the known Pacific haplotypes by building a median-joining haplotype network (<http://popart.otago.ac.nz/>, Bandelt *et al.*, 1999). We also ran one-level analyses of molecular variance (AMOVA; Excoffier *et al.*, 1992) in ARLEQUIN 3.5 (Excoffier & Lischer, 2010) on the data from the *Terrasses* and *ExBoDi* cruises to partition the molecular variance (1) among seamount and slope groups and (2) among depth groups (Kimura two-parameter distance matrix, gamma null, 10,000 permutations; Kimura, 1980).

RESULTS

Chrysogorgia biogeography on seamounts and slope of New Caledonia

Chrysogorgia was found on the slope of New Caledonia, on the NR and LR, and on the slope of MH, over most of the geographical and bathymetric range sampled during the *Terrasses* and *ExBoDi* cruises. Out of 261 stations sampled (99 and 162 for *Terrasses* and *ExBoDi*, respectively; Appendix S2), 74 recovered *Chrysogorgia*, 49 (66%) of which were sampled by dredging, reflecting the preference of *Chrysogorgia* for hard substrates. This proportion was consistent across slope and seamount stations. Over the 74 stations containing *Chrysogorgia* colonies, 31 haplotypes were detected from 71 stations (specimens from three stations could not be sequenced), eight of which were represented by single colonies (Appendix S1). Most haplotypes (52%) were represented by fewer than 10 specimens; 33 of the 71 stations contained multiple haplotypes. The most diverse stations (CP3898 and DW3855 on LR) contained 10 haplotypes. Most haplotypes

were found in the 200–600 m depth range, a bathymetric zone that has been extensively sampled (Fig. 3).

Of the 31 haplotypes, five were found exclusively on the slope, 16 exclusively on seamounts (19 if MH is included), and only six haplotypes were shared between the slope and the seamounts of the NR and LR (seven including MH); 20 haplotypes were sampled on LR, 9 of which were sampled nowhere else during the *Terrasses* and *ExBoDi* cruises. Only five haplotypes were found on both seamount chains. Seamount-level endemism was detected for 14 haplotypes, but five of these were observed only once each. Only three relatively well-sampled haplotypes were each restricted to one seamount (haplotypes 11, 24 and 27 on LR; $n \geq 20$). Haplotype 11 was sampled at especially great depths (750–990 m); if this taxon were indeed restricted to these depths, it could explain why we did not observe it elsewhere, most stations being shallower. Haplotypes 24 and 27, in contrast, were restricted to one seamount but were within the depth range of most sampling stations (240–345 m).

The abundances of the seven haplotypes shared between slopes and seamounts varied widely. To investigate whether species distributed across these habitats are found in different densities, we calculated the proportion of stations containing each of the seven haplotypes for slopes and

seamounts, and sorted these proportions into vectors of high-density and low-density occurrences. If haplotypes shared between these environments do not differ in their relative abundances, we expect the difference between these vectors to be non-significant. This was not the case (paired Wilcoxon signed rank test: $V = 0$, $P = 0.016$). For example, haplotype 23 was found at 5 of the 42 seamount stations where *Chrysogorgia* was collected and only at one station (out of 29) on the slope.

Seamount regions did not group together under hierarchical clustering. Rather, the slope region clustered with seamounts of the northern LR and MH, whereas the assemblage of the NR and southern LR grouped together. NMDS recovered a weak association of sites based on habitat (i.e. seamounts versus slope) (Fig. 4; ANOSIM R -statistic = 0.02, $P = 0.367$), and a stronger association of sites of similar depth (< 400 m, 400–600 m, ≥ 600 m; ANOSIM R -statistic = 0.22; $P = 0.048$).

Rarefaction curves suggest that seamounts are more haplotype-rich than the south-eastern slope of New Caledonia (Fig. 5). We observed 12 haplotypes in 181 individuals from 101 slope stations. For comparison, rarefaction of the seamount data recovered 18 haplotypes from 180 individuals, and 23 haplotypes from 101 stations. These richness estimates are relatively well supported statistically, as little (indi-

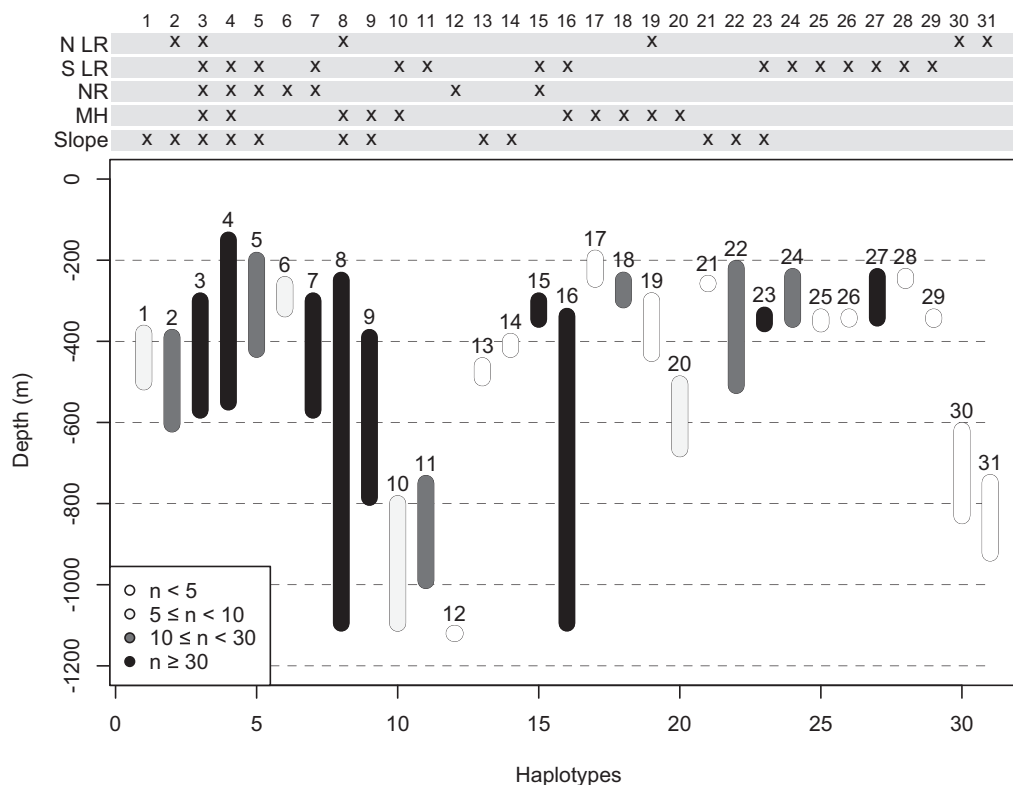


Figure 3 Geographical (top) and bathymetric (bottom) distributions of the 31 *Chrysogorgia* haplotypes sampled during the *Terrasses* and *ExBoDi* cruises in New Caledonia. Minimum and maximum depths were calculated based on the minimum and maximum depths of trawling and dredging stations that contained *Chrysogorgia*. The colour of each bar represents the number of colonies sampled for each haplotype (n ; see key in the bottom left corner). Haplotype number is provided on top of each bar. LR, Loyalty Ridge; NR, Norfolk Ridge; MH, Matthew and Hunter Islands.

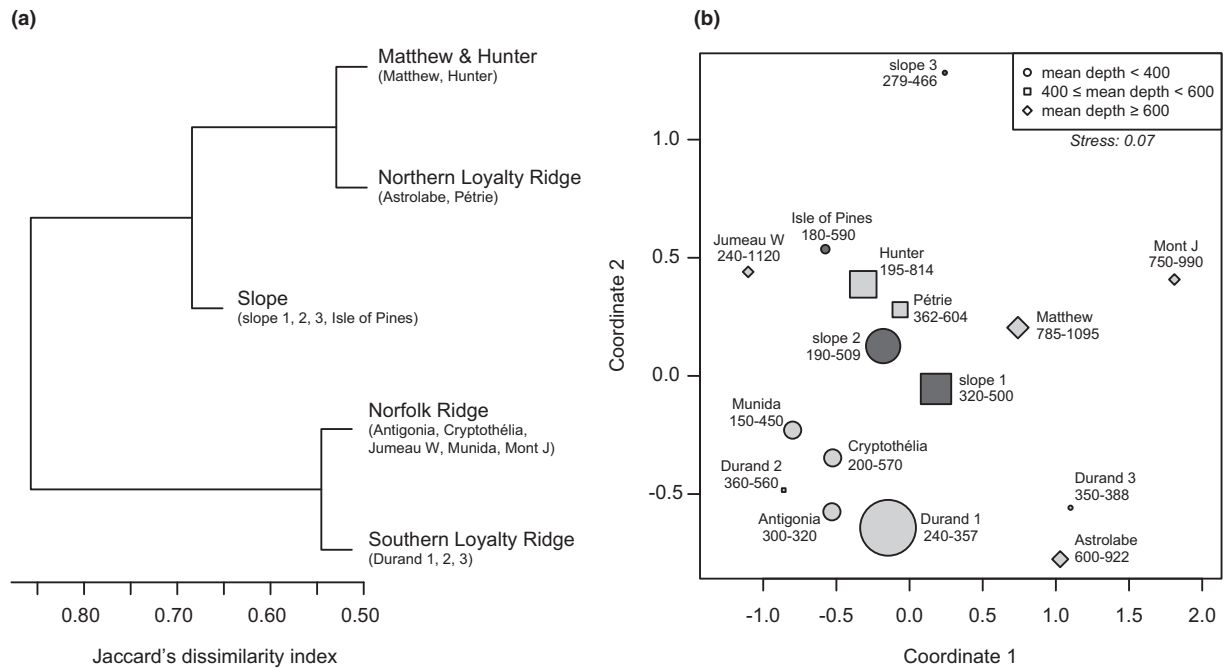


Figure 4 (a) Hierarchical cluster analysis and (b) non-metric multidimensional scaling (NMDS) based on Jaccard's dissimilarity between New Caledonian regions and sites. For NMDS, the symbol size is proportional to the number of *Chrysogorgia* haplotypes recorded at each site, and different symbols represent different mean depths (light grey, seamounts; dark grey, slope). Site names and depth ranges (m) are provided next to each symbol.

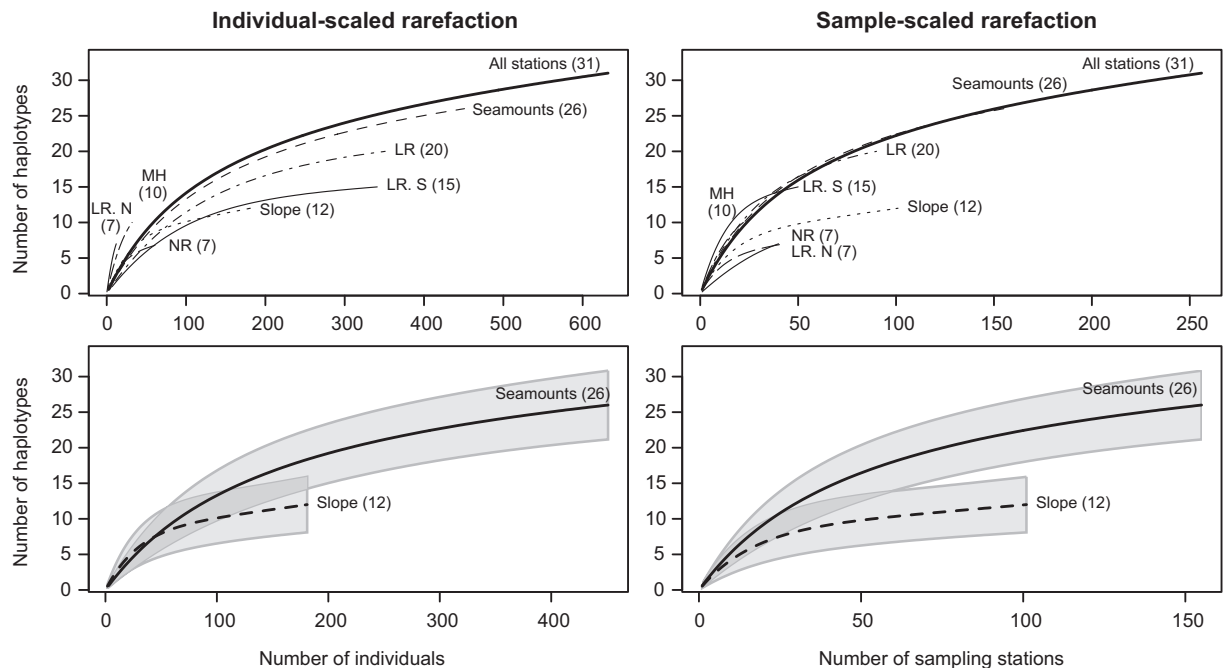


Figure 5 *Chrysogorgia* haplotype richness on the south-eastern New Caledonian slope and seamounts of the Norfolk Ridge (NR) and Loyalty Ridge (LR). Left: individual-scaled rarefaction curves. Right: sample-scaled rarefaction curves. Upper panels: rarefied haplotype richness for all regions. Lower panels: rarefied haplotype richness for seamounts and slopes, with 95% confidence envelopes. For each group, the total observed number of haplotypes is given in parentheses.

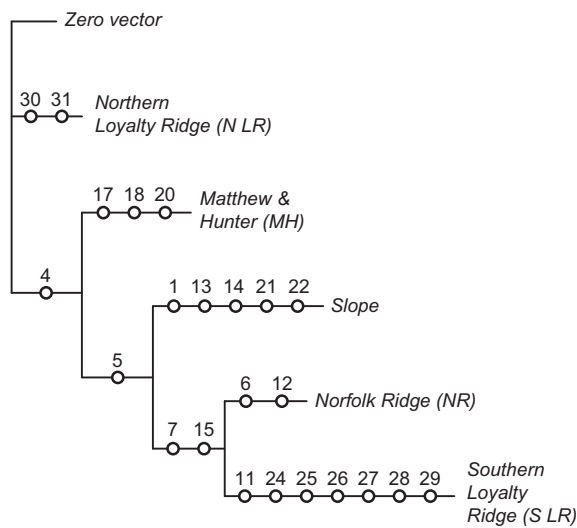


Figure 6 Parsimony analysis of endemism (PAE). The dots placed on branches of the tree represent *Chrysogorgia* haplotypes endemic to a clade (synapomorphic haplotypes), which can be defined by one or several New Caledonian regions.

vidual-scaled rarefaction) or no (sample-scaled rarefaction) overlap in 95% confidence intervals was observed. This pattern (seamounts being richer than the slope) is largely driven by the richness observed on southern LR seamounts (a total of 17 haplotypes). No rarefaction curve reached an asymptote, suggesting that more haplotypes would be discovered with additional sampling.

The most parsimonious tree produced by the PAE (score of 45, CI = 0.89, RI = 0.5) nested groups mostly according to geography (Fig. 6). PAE lumped stations on NR and the southern LR together, this clade comprising 11 of the 31 detected haplotypes (35%). The slope was sister to this clade, forming a group sheltering 17 endemics, i.e. over half of the total haplotype richness. Haplotype 3 was the only haplotype to be shared across all five regions, and seven haplotypes (2, 8, 9, 10, 16, 19 and 23) were shared by different clades (homoplastic haplotypes). Even though there are haplotypes shared between slope and seamounts, the haplotype network showed some evidence of evolutionary subdivision between these two groups (Fig. 7). This qualitative result was confirmed by the AMOVA, which suggests that the amount of genetic variance partitioned between groups (8.5%) is larger than expected by chance ($P = 0$). However, the amount of genetic variance explained by depth stratification was 3.4 times greater (variance component, 28.8%; $P = 0$; see Appendix S3).

Regional and global haplotype distributions

As reported in Thoma *et al.* (2009), the geographical distribution of haplotypes across the New England Seamounts (NES) and Corner Seamounts (CS) was accompanied by wide faunal connections with the Azores, the bathyal slope of the Bahamas, and Hawaii. Some haplotypes collected during the *Terrasses*

and *ExBoDi* cruises had wide distributions within the Pacific, but none of them were observed in the Atlantic. For example, eight haplotypes occurring in New Caledonia were collected in Papua New Guinea (PNG) (haplotypes 5, 7, 8, 9, 10, 16, 22 and 30; Pante *et al.*, 2012b). Three of these (haplotypes 7, 10 and 30) were collected on Sanguma Seamount (5.42° S, 154.02° E), the others coming from the PNG island slopes. None of these haplotypes were restricted to a single seamount peak, but four were restricted to seamounts (haplotypes 7, 10, 16 and 30; haplotype 22 being found exclusively on the slope, and haplotypes 5, 8 and 9 on both slope and seamounts). Haplotype 7, restricted to the seamounts of NR and LR, was also sampled on Nova Bank Seamount (780 km west of New Caledonia; 330–340 m depth), from a depth zone consistent with the bathymetric range observed on NR for this haplotype (300–390 m), deeper on a seamount off Taiwan (517–573 m), and on slopes and Sanguma Seamount in PNG (369–860 m). Similarly, a single colony of haplotype 12 was collected during the *Terrasses* and *ExBoDi* cruises. We found a specimen with an identical *mtMutS* sequence and congruent morphology from the south-west of the Kermadec Ridge (north-east of New Zealand). This haplotype is an example of a rare but widespread species. Interestingly, haplotype 9 appeared to have a very restricted distribution on the south-eastern slope of New Caledonia, but a specimen with an identical *mtMutS* sequence and with congruent gross morphology was sampled in the Solomon Islands during a TDSB cruise. This haplotype was also sampled in Hawaii (Middle Bank) and the Aleutian Islands (Alaska). The broad geographical distribution of some haplotypes points to the crucial role of sampling in assessing endemism. Haplotype 9, for instance, was the fourth most frequently collected taxon in New Caledonia, and was exclusively sampled on the south-western slope of the Isle of Pines ($n = 45$) and MH ($n = 1$). This haplotype was, however, also sampled in the Solomon Islands, over 1500 km from the Isle of Pines.

DISCUSSION

Correspondence between haplotypes and nominal species

A major issue for studies of coral biogeography and connectivity, this one included, is the difficulty of separating historical from contemporary connectivity. Wide geographical distributions could reflect either strong dispersal capabilities or ancient connections between ocean basins. We recently used genome-wide SNP (single nucleotide polymorphism) data to evaluate the hypothesis that mitochondrial *mtMutS* haplotypes can reliably represent species-level lineages, and found good congruence (67%) between *mtMutS*-based species delimitation and genomic divergence (Pante *et al.*, 2014). Specimens characterized by haplotypes 2, 8 and 13 may belong to a single species. Conversely, the New Caledonian specimens with haplotype 7 were phylogenetically distinct from the ones sampled in PNG, suggesting that colonies bearing this haplotype may

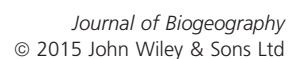


Figure 7 Median-joining network for all known Pacific *Chrysogorgia* haplotypes (a) and Venn diagram showing the haplotype distribution among Pacific regions (b). Network: mutational steps are represented by hashes on network branches; circle size is proportional to sample size. A detailed list of sampling locations and networks drawn according to depth and geography are available in Appendices S1 & S3 of Supporting Information.

belong to at least two species. Nevertheless, our simulation data (Appendix S2) suggest that, at the community level, underestimation of species richness due to lack of genetic resolution only has a moderate impact on the overall measure of faunal connectivity between slopes and seamounts. In addition, the apparent lack of geographical structuring on the NES and CS (Thoma *et al.*, 2009) is in sharp contrast with the data from New Caledonia. Our data are therefore useful in a comparative biogeographical framework.

Chrysogorgia on seamounts and slope

The apparent lack of endemism of chrysogorgiid corals on Atlantic seamounts is consistent with recent biogeographical and molecular studies of other seamount fauna (reviewed in Clark *et al.*, 2010; Rowden *et al.*, 2010a; Schlacher *et al.*, 2010). The overall consensus is that Pacific seamounts do not harbour significantly more endemic species than other, equivalent deep-sea habitats (Samadi *et al.*, 2006; O'Hara, 2007; Rowden *et al.*, 2010b). For instance, no endemism was detected among bamboo corals based on mitochondrial sequence data (Smith *et al.*, 2004). Similarly, Miller *et al.* (2010) recently reported on the genetic connectivity among nine species of corals (six scleractinians and three antipatharians) and found weak evidence for isolation by distance and barriers to dispersal between seamount peaks in the south-western Pacific. Our results are in contrast with those patterns, as up to 39% of *Chrysogorgia* haplotypes sampled more than once may be restricted to a single seamount. Durand Reef in particular appeared to be a hot-spot of diversity and endemism, with 13 haplotypes, half of which were observed nowhere else. The difference between our study and the studies of Smith *et al.* (2004) and Miller *et al.* (2010) may lie in the overall depth range surveyed; Miller and colleagues used specimens collected between 110 and 2136 m (median 542 m, mean 721 m), covering a much deeper range than we have here (70–1180 m; median 456 m, mean 497 m). The haplotypes reported by Smith *et al.* (2004) were also mostly restricted to waters deeper than 500 m, some extending as deep as 3000 m. These observations are consistent with the wide geographical distribution observed in the Atlantic, where sampling was mostly performed at depths between 1500 and 2000 m. In fact, Pante & Watling (2012) noted that none of the *Chrysogorgia* species previously detected on the slope of the north-western Atlantic were detected on the NES and CS, and that the sampling depth of these two species pools barely overlapped (see fig. 14 in Pante & Watling, 2012).

Geographical distribution structured by depth

The effect of depth on the distribution (reviewed in Etter & Rex, 2010) and genetic structure (e.g. France & Kocher, 1996; Zardus *et al.*, 2006; Cho & Shank, 2010; Jennings *et al.*, 2013) of deep-sea organisms has long been known, and is gaining recognition for corals (Eytan *et al.*, 2009; Baco & Cairns, 2012; Quattrini *et al.*, 2013, 2015; Doughty *et al.*, 2014). Environmental conditions such as pressure, temperature, dissolved

oxygen and habitat heterogeneity (to name a few; Gage & Tyler, 1991) can change significantly with depth, resulting in vertical zonation of benthic communities. These effects are, however, still little characterized for seamount fauna. McClain *et al.* (2010), investigating the structure of invertebrate and fish communities on Davidson Seamount in the north-eastern Pacific, found that assemblage composition, rather than diversity or density, changed with depth (1246–3656 m). In particular, they reported that octocorals showed significant species turnover between depths, which accounted for a significant proportion of the variation observed. In our study, differences in geographical and bathymetric haplotype distributions might be due to differences in the environmental setting, such as the sampled depth regimes, which overlapped only slightly (deepest NR/LR station: 1130 m; shallowest NES/CS station: 823 m). Etter *et al.* (2005) showed that genetic differentiation decreases with increasing depth in populations of deep-sea bivalves. Likewise, Bradbury *et al.* (2008) found a positive correlation between depth and pelagic larval duration, and a negative correlation between depth and amounts of genetic structure in marine fishes. Our results may therefore suggest that the stratification of *Chrysogorgia* haplotypes decreases with depth. This hypothesis, which implies that environmental conditions are less structuring at deeper depths, cannot be rigorously tested across ocean basins with the data at hand, but preliminary observations from our data suggest more complex patterns, because the deepest haplotypes observed on the LR and NR are not necessarily the most geographically widespread (e.g. haplotypes 12, 30 and 31, although sampling was less intensive below 800 m) and some shallow haplotypes are widely distributed (e.g. haplotypes 3 and 4; Fig. 3, Appendix S1). The stratification of haplotypes may therefore decrease below a depth of 1130 m; this could be tested by deeper sampling on the LR and NR.

Regional and global haplotype distributions

Chrysogorgia species pools from the Atlantic and Pacific Oceans seem to be characterized by different distributional ranges and diversity patterns. The *Terrasses* and *ExBoDi* haplotypes that were found outside the New Caledonian exclusive economic zone were mostly limited to the south-west Pacific region (from Taiwan to the Kermadec Ridge, a range of approximately 59° of latitude and 61° of longitude; with the exception of haplotype 9, the distribution of which extends to Hawaii). In contrast, all chrysogorgiid genera (*Chrysogorgia*, *Iridogorgia*, *Radicipes* and *Metallogorgia*) sampled on the Atlantic NES and CS contain haplotypes that are represented in the Pacific (Thoma *et al.*, 2009). In other words, the Atlantic chrysogorgiids appear to have an overall geographical range that is wider than that of New Caledonian chrysogorgiids. On the other hand, Pacific *Chrysogorgia* were characterized by high levels of patchiness, rarity and haplotypic diversity. Rarity and patchiness were observable at two levels. At the local scale, the number of colonies sampled at a single station was highly variable. For instance, at sta-

tions with *Chrysogorgia*, abundance varied from 1 to 109 colonies, with an average of 9 and a standard deviation of 18. At the regional scale, some haplotypes categorized as rare in New Caledonia were nevertheless sampled very far apart, such as in New Zealand, Papua New Guinea or Taiwan. These patterns of rarity and patchiness of deep New Caledonian fauna have also been observed in gastropods (Castelin *et al.*, 2011). These observations suggest that the sampling effort necessary to accurately describe the distribution of *Chrysogorgia* haplotypes in the Pacific is far greater than in the Atlantic. Nevertheless, sampling over 760 Pacific specimens provided data that emphasize the importance of depth, rarity and patchiness in structuring these communities, both on seamounts and on oceanic slopes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Excel table of *Chrysogorgia* specimens with biogeographical and haplotype information.

Appendix S2 Supplemental text.

Appendix S3 Median-joining network for Pacific *Chrysogorgia* haplotypes, plotted by depth and geography.

BIOSKETCH

Eric Pante and **Scott C. France** work on the evolution, biogeography and systematics of marine organisms, particularly deep-sea corals. **Sarah Samadi** and her group investigate faunal connectivity among benthic organisms of the deep tropical western Pacific Ocean.

Author contributions: E.P., S.C.F. and S.S. conceived the ideas; E.P., S.C.F. and S.S. (among others) collected the specimens; E.P., D.G. and C.C. generated the genetic data; E.P. analysed the data and wrote the paper; all authors approved the final version.

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**Investigation of selection pressures on ATP synthase genes across
a hybrid zone in the marine bivalve *Limecola balthica***

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Abstract

Little is known of the genes and gene interactions involved in inter-population genetic divergence in the context of secondary contact and local adaptation. We used transcriptome-wide DNA sequence data to describe patterns of divergence and selection across different populations of a marine bivalve (*Limecola balthica*). Our analyses focused on a nuclear gene (*atp5c1*) that was previously detected in an F_{ST} scan as highly structured across a contact zone on the Finistère Peninsula in France. This gene encodes the gamma subunit of the F_O/F_1 ATP synthase, a multi-protein complex that is particularly important in the metabolism of intertidal marine invertebrates. Analysis of non-synonymous to synonymous mutation ratios revealed that 65% of the gene is highly conserved ($dN/dS \leq 0.1$, $\min = 0$), while 6% of the gene is likely under positive selection ($dN/dS \geq 1$, $\max = 2.03$). All replacement mutations are clustered on a 46 residues portion of the protein, within an inter-peptide interaction zone. Comparative genomics suggests that these mutations are evolutionarily stable and are likely involved in inter-population genetic incompatibilities with the epsilon subunit of ATP synthase. The protein stability of the gamma subunit conferred by southern variants was inferred to be higher under warmer temperatures, suggesting that environmental conditions may contribute to the strength of intrinsic genetic barriers in *L. balthica*.

Keywords

Molecular evolution; hybridization; genetic incompatibilities; selection; ATP synthase; *Macoma balthica*

Running title

Barriers to gene flow in *Limecola balthica*

Introduction

Genetic divergence between populations can be caused and maintained by multiple factors, generally classified as exogenous and endogenous factors. While exogenous barriers to gene flow arise due to the maladaptation of phenotypes to varying environmental conditions (such as a temperature gradient), endogenous barriers involve negative interactions between alleles of different genetic backgrounds. Such negative interactions, formalized as Dobzhansky-Muller incompatibilities (DMIs, Dobzhansky, 1936; Muller, 1942), have long been recognized as important factor explaining the maladaptation of hybrid phenotypes (Presgraves, 2010; Maheshwari and Barbash, 2011). Incompatibilities between nuclear and organellar genes, a special case of DMI, are increasingly being recognized as an important factor maintaining genetic divergence between populations. In a recent review of mito-nuclear incompatibilities, Burton and Barreto (2012) argued that all aspects of mitochondrial functioning (translation, transcription, and replication of mtDNA, and ATP production through oxidative phosphorylation) may suffer from hybridization of diverging populations, because each of these activities relies on interactions between nuclear and mitochondrial genes.

Populations that diverged in allopatry and come into secondary contact are particularly prone to mito-nuclear incompatibilities (Burton and Barreto, 2012). In isolated populations, characteristics of the mitochondrial genome (generally: rapid evolution, recombination rare or absent, lower effective population size) can be fixed rapidly, and force tight co-adaptation with nuclear genes. Isolated populations that come into secondary contact may therefore no longer have compatible genetic backgrounds (Burton and Barreto, 2012). Populations that were historically isolated in glacial refugia may exhibit signatures of secondary genetic contact, and be geographically bounded by hybrid zones (Hewitt, 1996, 1999). These zones are therefore of prime interest for the study of DMI and mito-nuclear incompatibilities.

The Bay of Biscay, in the North Atlantic, is considered as a glacial refugium for many terrestrial (Gómez and Lunt, 2007) and marine (Maggs et al., 2008) species. Southern populations of *Limecola balthica*, a tellinid bivalve broadly distributed in the northern hemisphere (Väinölä and Varvio, 1989; Jansen et al., 2007), exhibit signatures of long-term isolation in the glacial refugium of the Bay of Biscay: high genetic diversity relative to previously glaciated areas, high prevalence of private alleles, and a sharp genetic break

separating them from northern populations (Becquet et al., 2012). Multiple genes involved in the oxidative phosphorylation (OXPHO) system (including genes coding for ATP synthase subunits and an ADP/ATP carrier) were detected as significantly differentiated among southern and northern populations by an F_{ST} scan (Pante et al., 2012). This observation, coupled with the strong differentiation of mitochondrial lineages suggested the potential role of mito-nuclear incompatibilities between individuals sampled south and north of the Finistère Peninsula (Pante et al., 2012). Furthermore, contrasted spatial patterns inferred from mitochondrial and nuclear markers (portion of the *cox1* gene and 8 microsatellites) also suggest that hybridization between divergent lineages occur (Becquet et al., 2012, and see Toews and Brelsford, 2012). If endogenous barriers are indeed involved in the differentiation observed between these populations, they may coincide with the exogenous barrier that constitutes the Finistère Peninsula, apparently inhospitable to *L. balthica*. The paucity of muddy sandflat habitats in this area results in low population densities (Becquet *et al.* pers. obs.) that may trap endogenous clines (Barton, 1979; Bierne et al., 2011; Abbott et al., 2013).

In this contribution, by re-analysis of the transcriptome data available for *Limecola balthica* (Pante et al., 2012), we suggest that mitochondrial function may indeed play a role in enforcing inter-population divergence by characterizing potentially adaptive genetic variation along the gene coding for the gamma subunit of the F_O/F_1 ATP synthase complex.

Material and Methods

Specimen sampling, and preparation of genetic data

Three geographically disjunct populations were sampled (Figure 1a, Pante et al., 2012). The sites of Aytré (Bay of Biscay, France; 46°13N 1°13W) and Somme Bay (English Channel, France; 50°21N, 1°62W) correspond to two discrete populations of the *Limecola balthica rubra* lineage (Becquet et al., 2012). Aytré is located near the southern limit of the species distributional range and is characterized warmer sea surface temperatures than the Somme Bay (Figure 1b). Furthermore, the Bay of Biscay is subjected to warming surface water temperatures (Goikoetxea et al., 2009). The third population, corresponding to the *L. balthica balthica* lineage, was sampled in Gdansk Bay (Baltic Sea, Poland; 54°21N 18°68E). cDNA libraries were

prepared from three pools of 10 individuals (one pool per site) and sequenced on a Roche 454 GS-FLX. Details on sequence quality control and assembly, read mapping, polymorphism detection, and gene annotation can be found in Pante et al. (2012). Here we focus on contig G_c113, detected in this latter study, and identified as coding for the gamma subunit of the ATP synthase F_1 rotor. We will refer to this gene as *atp5c1* (characterized in Matsuda et al., 1993).

Sequence evolution and predicted changes in protein function

atp5c1 was detected as potentially under strong selection using an F_{ST} scan, as 23/41 mapped SNPs were detected as statistical outliers (maximum $F_{ST} = 0.838$, Pante et al., 2012). The molecular signature of selection was investigated by looking at the ratio between non-synonymous (dN) and synonymous (dS) substitutions (e.g. Kimura, 1977), calculated using the R package *seqinr* (Charif and Lobry, 2007). A sliding window (150 nt wide, sliding every 3 nt) was used to detect variation in this ratio along the gene. dN/dS ratios were calculated based on population sequence consensus, as in Barreto et al. (2011). The programs SIFT (Kumar et al., 2009; Ng and Henikoff, 2006) and SNAP (Bromberg and Rost, 2007) were used to test whether variations in amino-acid (AA) compositions may have an impact on protein function. SIFT predicts whether AA substitutions can be tolerated by aligning and comparing homologous sequences retrieved by PSI-Blast; protein structure is not taken into account. SNAP uses sequence information, but can incorporate functional and structural annotations if such data is available (Bromberg and Rost, 2007). In SIFT, median conservation of sequences was set at 3.00 and sequences ≥ 90 % identical to the query were removed. The hypothesis that local environmental conditions, such as sediment temperature and pH, affect the stability of different alleles of *atp5c1* was tested using I-Mutant 2.0 (Capriotti et al., 2005). Protein stability ($\Delta\Delta G$) was estimated at 5, 15, and 30°C, and at pH 6, 7, and 8 (conditions based on observed data; Ekebom, 1999 and Lavergne and Barnett, personal communication). In order to further evaluate the impact of AA mutations on protein function, the structure of the gamma subunit was predicted using the I-TASSER server (Roy et al., 2010; Zhang, 2008) and visualized with the UGENE toolkit (Okonechnikov et al., 2012).

Results

Replacement mutations on *atp5c1*

Amino acid sequences of *atp5c1* from populations sampled some 2800 km apart are, overall, highly conserved, with a global dN/dS ratio of 0.08. The sliding window analysis (150 nt wide, sliding every codon; Figure 2a) revealed that 65% of the gene is under negative selection ($dN/dS \leq 0.1$, $\min = 0$), while 6% of the gene is under positive selection ($dN/dS \geq 1$, $\max = 2.03$). Five AA changes occur between residues 126 and 171, meaning that non-synonymous changes are clustered within a 46-residue fragment spanning 15% of the sequence. This region was predicted by I-TASSER (best model C-score = 0.04; Figure 2b) to overlap with two known peptide binding sites (Protein Data Bank IDs 2W6I and 2HLD; C-scores of 0.09 and 0.07, respectively). CDD annotation (Conserved Domains Database, Marchler-Bauer et al., 2013) following a BLASTX (Altschul et al., 1990) search for G_c113 suggest that these mutations occur at the interface with the core domain (interaction with the alpha-beta hexamere) and with the epsilon subunit. These non-synonymous changes were predicted to be evolutionarily tolerated based on SIFT and SNAP (expected SNAP accuracy 85-94%). The first four AA changes (L126A, N131D, D136N, H156D; nomenclature: Aytré considered as mutant) discriminate Aytré from Gdansk Bay and Somme Bay, while the fifth one (T171S) distinguishes Gdansk from the two French populations. N131D and H156D have a stabilizing effect on the protein, while all others have a destabilizing effect (I-Mutant tests between 15 and 30°C at pH7; Figure 3). In all cases, a relative increase in $\Delta\Delta G$ was observed with increasing temperature and pH (i.e., in warmer conditions, a destabilizing mutation was less destabilizing). In one case (N131D), $\Delta\Delta G$ changed sign (from negative to positive) with increased temperature (in all pH conditions), indicating that the expected effect of the mutation shifted from destabilizing to stabilizing. Considering the 4 AA mutations described above, the Aytré population contained a mix of two alleles, the major allele ($\sim 75\%$ of the reads) being unique to Aytré and one corresponding to the type found at Somme and Gdansk ($\sim 25\%$ of the reads). The dominant allele found in Aytré was not observed in Somme or Gdansk (median depth of coverage for G_c113 was 55, 45 and 18 for Aytré, Somme and Gdansk, respectively).

Potential for incompatibilities with other ATP synthase subunits

Contig G_c1077 was identified as coding for the alpha subunit and bear one outlier F_{ST} when comparing the samples from Aytré and Somme ($F_{ST} = 0.672$, Pante et al., 2012). This contig could be reliably placed in ORF, which revealed that all nine mutations were synonymous. All mutations segregated Aytré from Somme and Gdansk. Several contigs were identified as coding for the beta subunit; one (A_c2900) was included in our initial F_{ST} scan but did not stand out as bearing outlier SNPs (in addition, this config could not be reliably placed in ORF). Unfortunately, the epsilon subunit could not be detected in our transcriptome dataset.

Discussion

Intrinsic genetic incompatibilities among ATP synthase subunits

Intrinsic genetic incompatibilities (IGIs) can be significant post-zygotic barriers to gene flow (41% of 44 reviewed studies on reproductive barriers in plants and animals revealed IGIs, Nosil, 2012), yet few studies focus on marine model systems (e.g. two out the 20 species reviewed by Nosil *et al.* 2005). These species are, however, of particular interest, because they maintain large population sizes and are characterized by a strong dispersal potential that can prevent genetic differentiation over large geographic distances (e.g. Bierne et al., 2003), and they have proven valuable in understanding mechanisms of post-zygotic isolation (e.g. Palumbi 2009). The goal of this study was to further examine *atp5c1*, encoding the gamma subunit of the ATP synthase F_1 rotor, as it may be involved in IGIs (Pante et al., 2012). This gene is paramount to the good functioning of the F_O/F_1 complex, as its rotary action promotes ATP synthesis (reviewed in Sielaff and Börsch, 2013). Genetic incompatibilities between ATP synthase subunits, generally encoded by 12 nuclear and 2 mitochondrial genes (reviewed in Rand et al., 2004), have received attention as a potential mechanism leading to hybrid breakdown, enforcing barriers to gene flow between differentiated lineages (Gibson et al., 2010; Gagnaire et al., 2012; Burton and Barreto, 2012; Burton et al., 2013; Jacobsen et al., 2014). The gamma subunit is particularly noteworthy in this context, as its central position in the F_O/F_1 complex makes it interact with the beta and epsilon subunits of the F_1 portion, and the c subunits of the F_O portion (reviewed in Sielaff and Börsch, 2013). Our data suggest that five replacement mutations may interact with

other subunits, likely epsilon (encoded by *atp5e* in humans, Tu et al., 2000). This subunit sits on the F_O stator, which is composed of nuclear- and mitochondrial-encoded genes and is embedded in the mitochondrial membrane. While *atp5e* was not detected in our dataset, characterizing genetic variation at *atp5e* across the Finistère hybrid zone seems central to understanding how IGIs are involved in maintaining barriers to gene flow in *L. balthica*. The recent sequencing of mitochondrial genomes from individuals sampled on either sides of the Baltic and Finistère hybrid zones (Saunier et al., 2014) will help us shed some light on possible incompatibilities between the nuclear and mitochondrial genes coding for ATP synthase subunits. In addition, the recent discovery of sex-linked heteroplasmy in *L. balthica* strongly suggests that mitochondria in this species are characterised by doubly uniparental inheritance (Pante et al., 2017). In DUI species, the somatic tissues of males are characterised by a ‘female’ mitotype (as in all tissues of females), while the male germ line is characterised by a ‘male’ mitotype that is passed on from fathers to sons (reviewed in Zouros, 2013). In *L. balthica*, DMIs could therefore occur in multiple ways, as OXPHO epistatic interactions among nuclear genes, nuclear and female mitochondrial genes, and among nuclear and male mitochondrial genes in the sperm of interpopulational hybrids.

Asymmetric introgression across the Finistère hybrid zone

Considering the four amino acid sites segregating Aytré from Somme and Gdąnsk at *atp5c1*, two alleles were present at Aytré (75% of the allele unique to Aytré, and 25% of the allele shared with Somme and Gdąnsk), while the two other populations contained only a single allele (Figure 1c). We can therefore hypothesize an asymmetrical introgression of alleles from North to South. The same pattern of asymmetric allele distribution was observed at the mitochondrial *cox1* locus: northern *rubra* haplotypes penetrate into populations South of the Finistère Peninsula (prevalence 0 – 12.5%, median 4.1% across 8 sites), while the most common haplotypes South of the Finistère Peninsula were never sampled North of Brittany (Becquet et al., 2012). Patterns of asymmetric introgression of alleles are commonly observed in hybrid zones (Barton and Hewitt, 1985), and were observed in the Bay of Biscay between *Mytilus edulis* and *M. galloprovincialis* (Rawson and Hilbish, 1998). Gagnaire et al. (2012), looking at divergence and selection among populations of the eels *Anguilla rostrata* and *A. anguilla* (two hybridizing sister species) also found evidence of unidirectional

introgression from *A. rostrata* to *A. anguilla* along part of the *atp5c1* locus. Whether asymmetric clines in *L. balthica* are due to cline movement, differential gene flow, population structure, or selection (Barton and Hewitt, 1985) is avenue for future research. In particular, asymmetrical introgression across the Finistère hybrid zone calls for a test of asymmetric fitness of inter-lineage crosses (e.g. Turelli and Moyle, 2007). An involvement of mitochondrial genes in incompatibilities among F_0/F_1 subunits could explain asymmetric allele frequencies observed, if northern hybrids with southern mitochondria are less fit than southern hybrids with northern mitochondria.

Adaptive divergence at OXPHO loci

Mitochondrial function can be significantly influenced by temperature (e.g. Dahlhoff and Somero, 1993). In Dutch populations of *L. balthica*, respiration rate was shown to increase with temperature intra-seasonally (Hummel et al., 2000). In addition, Dutch specimens transplanted in the Spanish estuary of Bidasoa, 200 km south of the known species range limit (i.e. the Gironde Estuary, some 80 km from our sampling site in Aytré, France) showed respiration rates significantly higher than in their native populations (Hummel et al., 2000). Our analyses on the effect of temperature on the stability of subunit gamma suggest a small, positive effect of each replacement mutation in the southern population. While only five replacement mutations were detected, previous studies have shown that even few differences in amino acid composition can have a significant impact on mitochondrial performance. In the marine copepod *Tigriopus californicus*, three amino acid changes at the nuclear cytochrome *c*, separating populations characterized by different thermal regimes, correlated with significant differences in cytochrome *c* oxidase activity (Rawson and Burton, 2002). In the seed beetle *Callosobruchus maculatus*, metabolic rates were detected between cytotypes that differed by one amino acid at COI and one amino acid at Cyt-B (Arnqvist et al., 2010, and see below). In killer whales (*Orcinus orca*), single amino acid changes at Cyt-B are associated with Antarctic ecotypes, and are therefore possibly implicated in improved mitochondrial performance in polar waters (Foote et al., 2011). Cyt-B was also recently implicated in thermal adaptation in the European anchovy (Silva et al., 2014). Finally, the synergistic impact of temperature and few replacement mutations on protein stability and function was recorded at the nuclear locus encoding cytoplasmic malate dehydrogenase (cMDH) in

limpets (Dong and Somero, 2009). As the surface water of the Bay of Biscay is known to be warming at rate of 0.26°C/decade (1977-2007 time period, Goikoetxea et al., 2009) and the geographical range (Jansen et al., 2007) and phenology (Saunier et al., submitted) of southern *L. balthica* are both currently receding, it becomes increasingly important from a conservation standpoint to understand how genetic diversity at OXPHO genes relates to adaptive potential.

Interplay between intrinsic genetic incompatibilities and temperature

One fascinating avenue for research is the characterization of interactions between intrinsic genetic incompatibilities and extrinsic environmental forces. Arnqvist et al. (2010) were the first to demonstrate environmental effects on mitonuclear epistatic interactions by crossing mitochondrial and nuclear genomes of *C. maculatus*. In this very elegant experiment, the authors showed that the negative effects of genetic incompatibilities on metabolic rate were only detectable when hybrids were exposed to different temperatures (Arnqvist et al., 2010). In *L. balthica*, the replacement mutations mapped on *atp5c1* (i) are located in an inter-peptide interaction zone (most likely in the area where the gamma subunit interacts with epsilon), and (ii) influence the stability of the gamma subunit depending on temperature and pH. The F_O/F₁ ATP synthase complex of *L. balthica* is therefore a noteworthy model to study how temperature can influence mitonuclear epistatic interactions and enforce genetic barriers in a high gene flow marine species.

Conclusion

Re-analysis of transcriptome data from *L. balthica* for the nuclear gene *atp5c1*, encoding the gamma subunit of the mitochondrial F_O/F₁ ATP synthase complex, brought forth a high dN/dS ratio corresponding to a discrete, 46-residue long portion of the protein. The five replacement mutations enclosed in this region are bracketed by known, conserved inter-peptide interaction sites, and are likely involved in inter-population genetic incompatibilities with the epsilon subunit of ATP synthase. The inferred protein stability conferred by these mutations under warmer temperatures suggests that environmental conditions may contribute to the strength of intrinsic genetic barriers in *L. balthica*.

Data accessibility

DNA sequences: Genbank accessions xxxxxxxx-xxxxxxx.

Competing interests

We have no competing interests.

Authors' contributions

All authors designed this experiment. VB acquired the data. EP analyzed the data and wrote the manuscript.

VB and PG revised the manuscript and gave final approval for publication.

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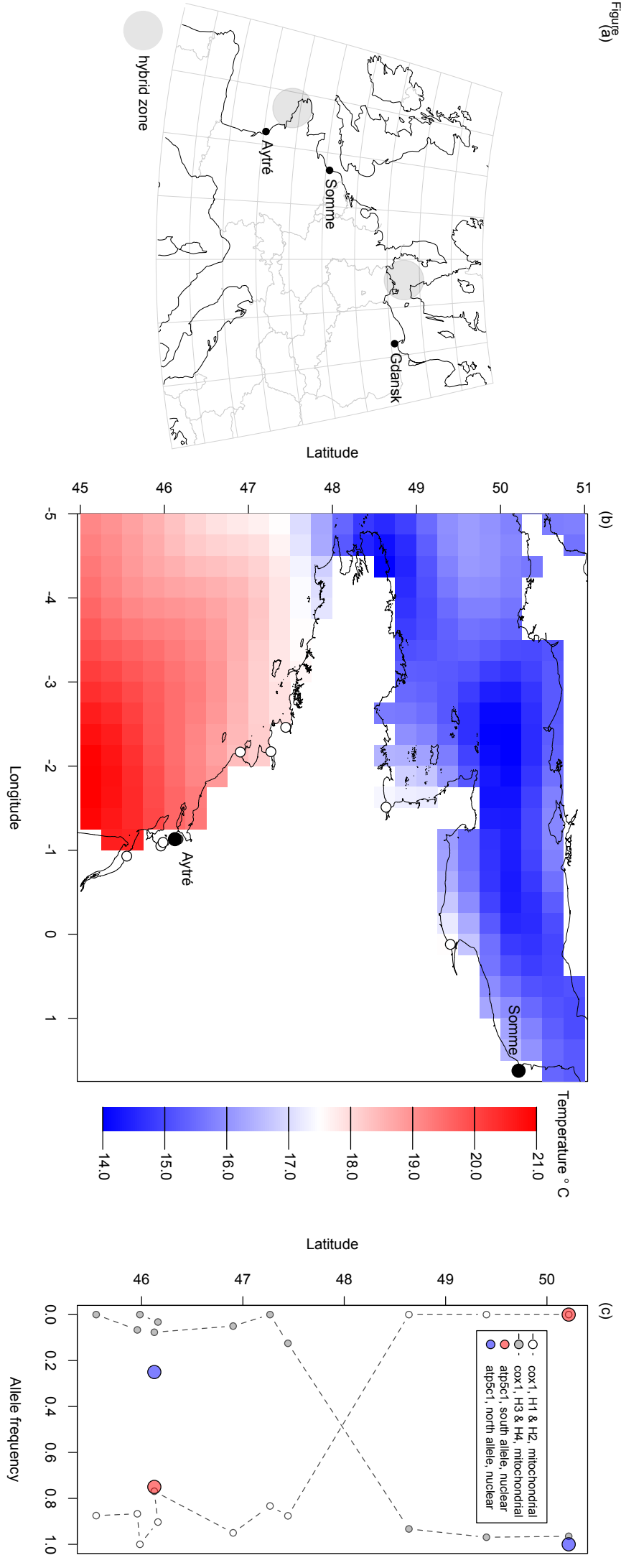
Figure captions

Figure 1. (a) Sampling map displaying the location of two hybrid zones. (b) Heat map of the Bay of Biscay and English Channel with sampling sites of this study (black dots) and of Becquet *et al.* (2012)(white dots). SST are monthly averages for July, for 1982, 1992, 2002 and 2012; high Resolution SST data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA (Reynolds et al., 2007). (c) Mitochondrial haplotype frequencies at *cox1* (data from Becquet et al., 2012, site locations on panel b) and allele frequencies at the nuclear *atp5c1* (this study).

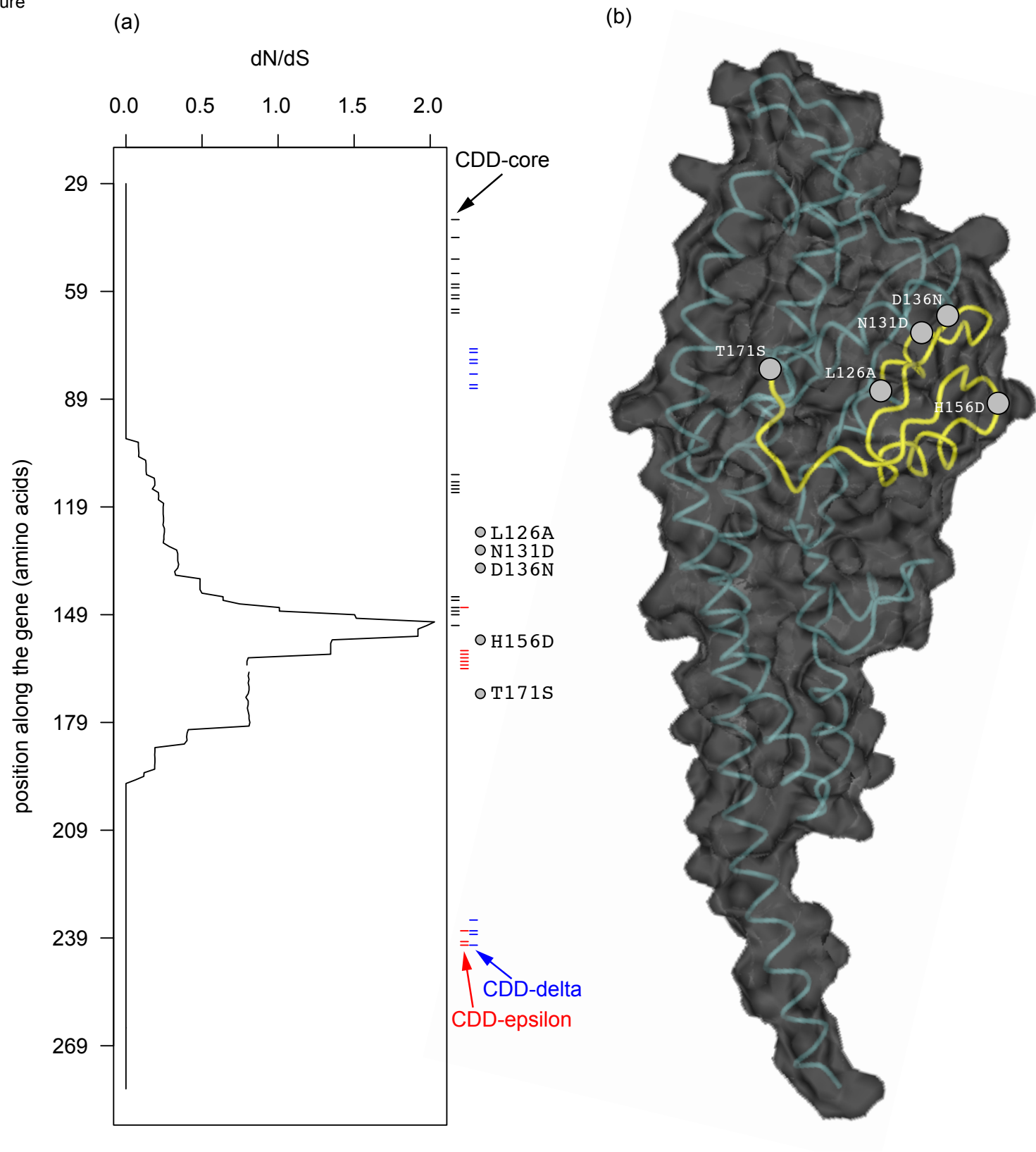
Figure 2. (a) Sliding window analysis of the dN/dS ratio along *atp5c1* (window 50 AA wide, sliding every codon). The position of replacement mutations is provided on the right (grey dots), as well as the position of conserved interaction sites detected by CDD (Marchler-Bauer et al., 2013) (black, blue and red: interactions with core domain, delta and epsilon subunits, respectively). (b) Three-dimensional model of the ATP synthase gamma subunit of *Limecola balthica* (I-TASSER prediction based on reference contig from Gdansk). The yellow segment represents the 46 residue-long fragment bearing replacement mutations (grey dots).

Figure 3. Effect of temperature and pH on the stability (represented as $\Delta\Delta G$) of the ATP synthase gamma subunit. Error bars represent one standard deviation of three measures of $\Delta\Delta G$ per temperature, using different pH values (see methods). The name of replacement mutations is provided on the right.

Figure (a)



Figure



Figure

