Molecular Phylogenetics and Evolution 57 (2010) 71–83

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

New insights into diversity and evolution of deep-sea Mytilidae (Mollusca: Bivalvia)

Julien Lorion [a,*], Barbara Buge [c], Corinne Cruaud [b], Sarah Samadi [a]

[a] Muséum National d'Histoire Naturelle, UMR 7138 UPMC-IRD-MNHN-CNRS 'Systématique Adaptation Evolution', Equipe 'Espèces et Spéciation', 57 Rue Cuvier CP 26, F-75231 Paris Cedex 05, France
[b] GENOSCOPE, 2 rue Gaston Crémieux, CP 5706, 91057 Evry Cedex, France
[c] Muséum National d'Histoire Naturelle, Direction des collections, 55 rue Buffon, CP 51, F-75231 Paris Cedex 05, France

ARTICLE INFO

Article history:
Received 12 October 2009
Revised 26 May 2010
Accepted 29 May 2010
Available online 15 June 2010

Keywords:
Phylogenetics
Bathymodiolinae
Hydrothermal vent
Cold seep
Idas
Adipicola
Organic falls
South-West Pacific
Taxon sampling
Allopatry

ABSTRACT

Bathymodiolinae mussels have been used as a biological model to better understand the evolutionary origin of faunas associated with deep-sea hydrothermal vents and cold seeps. Most studies to date, however, have sampled with a strong bias towards vent and seep species, mainly because of a lack of knowledge of closely related species from organic falls. Here we reassess the species diversity of deep-sea mussels using two genes and a large taxon sample from the South-Western Pacific. This new taxonomic framework serves as a basis for a phylogenetic investigation of their evolutionary history. We first highlight an unexpected allopatric pattern and suggest that mussels usually reported from organic falls are in fact poorly specialized with regard to their environment. This challenges the adaptive scenarios proposed to explain the diversification of the group. Second, we confirm that deep-sea mussels arose from organic falls and then colonized hydrothermal vents and cold seeps in multiple events. Overall, this study constitutes a new basis for further phylogenetic investigations and a global systematic revision of deep-sea mussels.

1. Introduction

Phylogenetic studies based on sampling that does not reflect the breadth of species diversity across ecosystems may lead to biased conclusions about their evolutionary history. However, prior knowledge regarding the possible relationships among ecosystems and the species diversity that should be sampled to properly address evolutionary issues are not always available. Evolutionary studies focusing on evolutionary relationships among organisms from deep-sea ecosystems often suffer from this problem (Samadi et al., 2006; McClain, 2007; O'Hara, 2007; Vrijenhoek, 2009).

In this regard, the case of hydrothermal vent and cold seep faunas is striking. Being the first discovered of chemosynthesis-based ecosystems, vents and seeps are one of the most amazing findings in oceans in the last 40 years (Cavanaugh et al., 2006; Dubilier et al., 2008; Lonsdale, 1977). As a consequence, both environments have been extensively studied and much data are now available regarding vent and seep ecology, as well as the physiological features of organisms living there (Hourdez and Lallier, 2006; Sibuet and Olu, 1998; Van Dover, 2000). But in contrast, their evolutionary origins are less well documented. Distel et al. (2000) nevertheless showed the close relationship between symbiont-bearing mussels of the genera *Bathymodiolus* Kenk and Wilson 1985 and *Tamu* Gustafson, Turner, Lutz and Vrijenhoek 1998 (Mytilidae: Bathymodiolinae), endemic to vents and seeps, and the small-sized mussels of the genera *Idas* Jeffreys 1876, *Adipicola* Dautzenberg 1927 and *Benthomodiolus* Dell 1987 (Mytilidae: Modiolinae), usually associated with shallower and more poorly known organic fall ecosystems. These mainly consist in sunken wood and vegetal debris but also include vertebrate bones. While decomposing at the deep-sea floor, organic falls produce sulfide that is used by mussels through chemosynthetic symbionts similar to those of vent and seep mussels (Duperron et al., 2009).

Distel et al. (2000) therefore clarified an important issue: understanding of the evolutionary history of vent and seep mussels requires knowledge of their relationships with relatives from organic falls. They additionally proposed the "wooden-step" hypothesis, which suggests that the mussels from vents and seeps evolved recently from ancestors associated with organic falls. Other data confirmed that many vent faunas are much younger than previously thought (Little and Vrijenhoek, 2003), and challenged previous hypotheses that suggested that hydrothermal vents had experienced "a long and continuing evolutionary history" (Newman, 1985). However, the "wooden-step" hypothesis proposed by Distel et al. (2000) relies more on ecological and

* Corresponding author. Fax: +33 (0) 1 40 79 38 44.
E-mail address: lorion@mnhn.fr (J. Lorion).

1055-7903/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2010.05.027

paleontological data (Kiel and Goedert, 2006a; Smith and Baco, 2003) than on a reliable phylogenetic reconstruction. Indeed the 18S rRNA-based tree provided in their study was poorly resolved. More recent studies investigating the evolutionary origin of vent and seep mussels used more rapidly evolving genes and/or more taxa (Jones et al., 2006; Samadi et al., 2007; Lorion et al., 2009; Fujita et al., 2009). But at most 6 species from organic falls were used in these studies, out of at least 20 reported in the taxonomic literature (Dell, 1987; Warén and Carrozza, 1990). This is because most species were described long time ago: their soft tissues are lacking or not very suitable (formalin-fixed) for extensive molecular studies. New samples are thus strongly needed. As organic falls are unevenly distributed at the deep-sea floor and often thinly dispersed, sampling strategies different from those used for the exploration of vents and seeps are also required.

Once solved, the issue of sampling leads to other difficulties related to the poor taxonomic knowledge of mussels associated with organic falls. Indeed, many species were described on the basis of a few shell characters, providing few diagnosable characters (see however Dell, 1987 and Gustafson et al., 1998). The species descriptions were often based on a few specimens and thus generally without description of life stages, levels of environmental plasticity and any other details of polymorphism that characterize species. As a consequence, some species descriptions are ambiguous and the distinction between the genera *Idas* and *Adipicola* is debated (Gustafson et al., 1998; Warén, 1993). Therefore, the methods for species delimitation and the potential extent of diversity in mussels from organic falls would greatly benefit from a complete reassessment. This task is made difficult by several issues related to the evolutionary significance of shell characters classically used for alpha-taxonomy in molluscs (Knowlton, 2000; Vrijenhoek, 2009). Indeed, shell morphology in molluscs is subject to crypticism (Won et al., 2003b; Lee and Foighil, 2004; Johnson et al., 2009), environmental plasticity (Yeap et al., 2001; Wullschleger and Jokela, 2002; Baker et al., 2003; Hollander et al., 2006; Pfenninger et al., 2006) and growth allometry (Horikoshi and Tsuchida, 1984). By contrast, using anatomical characters is often more useful in species delimitations (e.g. Cosel, 2008). However, especially when specimens are small (mussels from organic falls are usually less than 1 cm length), it is difficult and time consuming to analyze anatomical characters for many specimens. Moreover anatomy may not solve the problem of cryptic species.

In this context, it seems relevant to first use molecular characters for species delimitation (Blaxter, 2003; Blaxter, 2004; Vogler and Monaghan, 2007). From a given set of specimens, primary hypotheses of species delimitation can be drawn, for instance, using a criterion of similarity based on genetic distances. In an integrative framework (De Queiroz, 2007; Samadi and Barberousse, 2009), these primary hypotheses can be refined using the alternative criterion of monophyly (Meier and Willman, 2000; Sites and Marshall, 2004; Wheeler and Meier, 2000) and/or additional unlinked markers. The analysis of unlinked markers moreover allows evaluating the occurrence of gene flow between putative species, and thus to use the additional criterion of reproductive isolation (Taylor et al., 2000). Interestingly, recent methodological developments, rooted in the methodological framework of the coalescent theory, offer reliable opportunities to use simultaneously several of these criteria (Nielsen et al., 2001; Pons et al., 2006). Finally, the molecular hypotheses of species delimitation should be discussed using ecological and morphological data (Will and Rubinoff, 2004; Will et al., 2005).

To fulfill the sampling needs required to document the evolutionary history of deep-sea mussels, specimens associated to organic falls were collected throughout the South-West Pacific, one of the most species-rich area of the marine environments. Here, an integrative approach to taxonomy based on the analysis of two unlinked gene fragments is used to overcome the poor evolutionary significance of shell characters. Both the sampling effort and the integrative approach provide a new taxonomic framework for the mussels associated to organic falls. This then serves as a basis for investigation of the diversification processes of mussels in the deep-sea. Hypotheses regarding the speciation patterns in mussels from organic falls are formulated and constitute first insights regarding biogeography in these environments. Relationships among mussels from organic falls with mussels from vent and seep allow discussion of various aspects of the "wooden-step" hypothesis such as the connectivity among habitats and the relative roles of adaptive and stochastic processes in the species diversification. Finally, implications of our results for further investigations of the systematics of deep-sea mussels are evaluated.

2. Material and methods

2.1. Sampling

Most of the new material studied herein was collected during cruises of the Tropical Deep-sea Benthos program from 1999 to 2006 (Bouchet et al., 2008). Samples from these cruises include 212 mussels collected off the Philippines (137 specimens), Vanuatu (58 specimens), Solomon Islands (9 specimens), Chesterfields Islands (4 specimens), and Fiji (4 specimens) (Fig. 1). These new samples represent a total of 18 localities and 59 stations trawled at depth ranging from 103 m to 1392 m (Supplementary data 1 and 2). Moreover two colonization modules were deployed off Noumea (New Caledonia) in October 2003 and September 2005

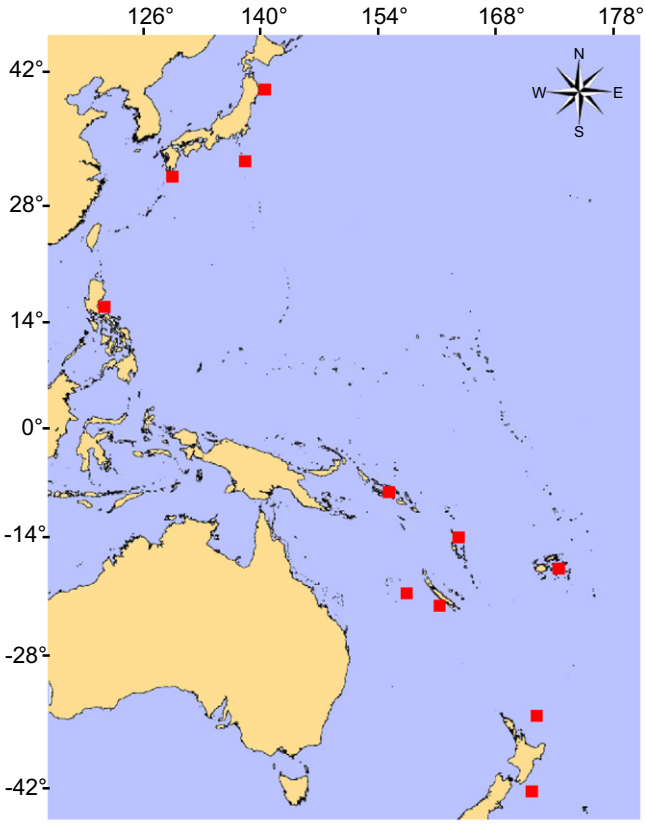

Fig. 1. Western Pacific map showing the distribution of localities (squares) from which mussels associated with organic falls were available for this study, taking into account new samples and data from Genbank.

at 1060 m and 800 m depth, respectively. These were recovered in October 2005 and May 2008, respectively, and allowed collection of 5 and 28 specimens, respectively, from turtle bones. Upon recovery, all samples were immediately stored in 90% EtOH. Collaborators kindly provided additional alcohol-fixed specimens from organic falls, including four paratypes of *Adipicola arcuatilis* (NMNZ M. 089789) collected from whale skull off New Zealand and three specimens collected from sunken wood off Japan. All these new specimens were deposited both in collections of the Muséum National d'Histoire Naturelle (MNHN) and in the Barcoding Of Life Database (BOLD, project WFAL).

Tissues from the 16 specimens analyzed by Samadi et al. (2007), from the coastal species *Modiolus modiolus*, from the seep species *Bathymodiolus mauritanicus*, *Ba. boomerang* and *Idas* sp. Med (undescribed species), and from the vent species *Ba. thermophilus, Ba. manusensis, Ba. taiwanensis* and NZ3 (undescribed species) were available at the MNHN and added to our new samples (Tables 1 and 2). Genus names will be abbreviated hereafter as following: *M. Modiolus*, *Be. Benthomodiolus*, *I. Idas*, *A. Adipicola*, *Ba. Bathymodiolius*, *G. Gigantidas*, and *T. Tamu*.

2.2. Molecular methods

DNA was extracted from whole specimens or gills of largest specimens using the QIAamp® DNA Micro Kit (Qiagen). A fragment of the cytochrome oxidase I (COI) mitochondrial gene was amplified using the primers H691 (5′-GTRTTAAARTGRCGATCAAAAAT-3′), which was designed for deep-sea mussels, and LCO1490 (Folmer et al., 1994). Domains D1, D2 and D3 (Qu, 1986) of the 28S rRNA nuclear gene were amplified using primers C1′ (5′-ACCCGCTGAATTTAAGCAT-3′) and C4 (5′-TCGGAGGGAACCAGCTACTA-3′). PCR reactions were performed in 25 μL final volume, containing approximately 3 ng template DNA, 1.5 mM $MgCl_2$, 0.26 mM of each nucleotide, 0.3 μM of each primer, 5% DMSO and 0.75 U of Taq Polymerase (Qbiogene). Amplification products were generated by an initial denaturation step of 4 min at 94 °C followed by 35 cycles at 94 °C for 40 s, 50 °C (52 °C for 28S rDNA) for 50 s and 1 min at 72 °C, and by a final extension at 72 °C for 10 min. PCR products were purified and sequenced with the PCR primers at the Genoscope (Evry) in both directions. Chromatograms were edited using Sequencher 4.1.4 and sequences were aligned using the Clustal W module included in Mega 4.0 (Tamura et al., 2007). The sequences of newly sampled specimens were deposited both in BOLD and in Genbank (COI mtDNA: FJ937033–FJ937283, GU073224; 28S rRNA: GU065746-GU065881).

2.3. An integrative approach to alpha-taxonomy

The purpose of this integrative approach to alpha-taxonomy is to use all available sources of evidences to propose hypotheses of species delimitation. Classically in malacology the primary hypotheses of species delimitations are drawn from morphology and then documented using other lines of evidence. We here built primary hypotheses upon molecular characters and then discussed these hypotheses in the light of other evidences.

The COI mtDNA encoding-gene was used to propose primary hypotheses of species delimitations. In that purpose we used the General Mixed Yule Coalescent model (GMYC, single threshold algorithm) (Fontaneto et al., 2007; Monaghan et al., 2009; Pons et al. 2006). The aim of this method is to classify the branching time intervals defined by the nodes in an ultra-metric tree to either being the result of inter-specific or intra-specific processes of lineage branching. The main GMYC parameters are the threshold between coalescent and speciation branching times, the coalescent branching rate and the species branching rate. Two additional scaling parameters are used to relax strict assumptions of constant population size and speciation rates. The likelihood function derived from this model is optimized across an input tree. The method allows detecting monophyletic entities that may be

Table 1
Species name, substrate, geographical location and Genbank accession numbers of specimens associated with organic falls previously published and used in this study. The number of sequences is given within brackets when higher than one. Authors of sequences: [1]Fujita et al. (2009), [2]Smith et al. (2004), [3]Jones et al. (2006), [4]Samadi et al. (2006), [5]Iwasaki et al. (2006), [6]Lorion et al. (2009), [7]Duperron et al. (2008a).

Species	Substrate	Locality (country)	COI mtDNA	28S rRNA
Be. geikotsucola Okutani and Miyazaki 2007	Bone	Torishima seamount (off Japan)	AB257513 and AB257514 (5)[1]	
Be. lignicola Dell 1987	Wood, bone	Chatham Rise (off New Zealand)	AY275545(1)[2]	AY781131[3]
A. longissima Thiele and Jaeckel 1931	Wood	Solomon islands, Philippines	EU350072, DQ340773, DQ340777, DQ340783, DQ340784, DQ340788, DQ340789, DQ340791, DQ340792 (9)[4–7]	DQ863945[4]
A. pacifica Dall, Bartsch and Rehder 1938	Bone	Off Noma cape (Japan)	AB257526–AB257528, AB170040 (7)[1–5]	
A. crypta (B′) Dall, Bartsch and Rehder 1938	Wood, bone	Vanuatu	EU702316–EU702319, EU702321 (5)[6]	EU683296–EU683300 (4)[6]
A. crypta (B″) Dall, Bartsch and Rehder 1938	Wood, bone	Off Noma cape (Japan) Philippines Vanuatu	EU702315, EU702320, AB257515–AB257519 (14)[1–6]	EU683297, EU683301 (2)[6]
A. iwaotakii Habe 1958 (A′)	Wood	Off Hitachinaka city (Japan) Vanuatu	AB257520, AB257521, AB257523, EU702323 and EU702324, EU702326–EU702349 (29)[1–6]	EU683282–EU683294 (13)[6]
A. iwaotakii (A″) Habe 1958	Wood	Off Hitachinaka city (Japan) Philippines	EU702322, EU702325 (2)[6]	EU683295[6]
I. japonica Habe 1976	Wood	Off Noma cape (Japan)	AB257536–AB257537 (2)[1]	
I. washingtonia Bernard 1978	Wood, bone	Monterey Bay (USA)	AY275546(1)[2]	AY781146[3]
Idas sp. SAL1	Wood	Solomon islands	DQ340775, DQ340778, DQ340780, DQ340782, DQ340785, DQ340787, DQ340790 (7)[4]	DQ863944[4]
Idas sp. SAL3	Wood	Solomon islands	DQ340772, DQ340774, DQ340781 (3)[4]	DQ863946[4]
Idas sp. SAL4	Wood	Philippines Solomon islands	DQ340776, DQ340779, DQ340786, DQ340793 (4)[4]	DQ863947[4]
Idas sp. *C*	Wood, bone	Solomon islands Vanuatu	EU702360–EU702377 (18)[6]	EU683259–EU683272 (14)[6]
Idas sp. *D*	Wood	Philippines	EU702350–EU702359 (10)[6]	EU683273–EU683281 (9)[6]

Table 2
Names, habitat, depth range, geographical location of species from hydrothermal vents, cold seeps and coastal environments used in this study. Genbank accession numbers are given. Geographical locations are abbreviated as following: GoM: Gulf of Mexico, NA: Northern Atlantic, WP: Western Pacific, EM: Eastern Mediterranean. The authors of sequences: [1]this study, [2]Samadi et al. (2006), [3]Jones et al. (2006), [4]Won et al. (2003), [5]Smith et al. (2004), [6]Miyazaki et al. (2000), [7]Duperron et al. (2008b).

Species	Habitat	Depth range	Locality	COI mtDNA 28S rRNA
M. modiolus Linné 1758	Intertidal Subtidal	10–300 m	Ireland sea (NA)	FJ890501[1] EF526455[2]
Ba. brooksi Gustafson, Turner, Lutz and Vrijenhoek 1998	Seep	2.222–3.314 m	West Florida Escarpment (GoM)	AY649798[3] AY781135[3]
T. fisheri Gustafson, Turner, Lutz and Vrijenhoek 1998	Seep	546–650 m	Garden Banks (GoM)	AY649803[3] AY781132[3]
Ba. heckerae Gustafson, Turner, Lutz and Vrijenhoek 1998	Seep	3.314 m	West Florida Escarpment (GoM)	AY649794[3] AY781138[3]
Ba. azoricus Cosel and Comtet 1999	Vent	866–2.330 m	Menez Gwen (NA)	AY649795[3] AY781148[3]
Ba. puteoserpentis Cosel, Metivier and Hashimoto 1994	Vent	3.023–3.510 m	Snake Pit (NA)	AY649796[3] AY781151[3]
Ba. thermophilus Kenk and Wilson 1985	Vent	2.460–2.747 m	Eastern Pacific Rise	GU966639[1] GU966640[1]
Ba. aff. *thermophilus*	Vent	2.331 m	Eastern Pacific Rise	AF456317[4] AY781140[3]
G. gladius Cosel and Marshall 2003	Vent	300–460 m	Rumble III (WP)	AY649802[3] AY781134[3]
Ba. tangaroa Cosel and Marshall 2003	Vent	920–1.205 m	Cape Turnagain (WP)	AY608439[3] AY781149[3]
Ba. brevior Cosel, Metivier and Hashimoto 1994	Vent	3589 m	Mariana Trough (WP)	AY649799[3] AY781150[3]
Ba. mauritanicus Cose2002	Seep	540–2.222 m	Barbados Accretionary Prism (NA)	FJ890502[1] FJ890504[1]
Ba. taiwanensis Cosel 2008	Seep	200–355 m	Okinawa arc (WP)	GU966638[1] GU966641[1]
Ba. boomerang Cosel and Olu 1998	Seep	1.000–3.170 m	Barbados Accretionary Prism (NA)	FJ890503[1] FJ890505[1]
Ba. aduloides Hashimoto and Okutani 1994	Vent	1.378–1.451 m	Iheya Ridge (WP)	AB170054[6]
Ba. manusensis Hashimoto and Furuta 2007	Vent	1.627 m	Manus basin (WP)	GU966637[1] GU966642[1]
NZ3	Vent	200 m	Macauley Come (WP)	FJ767936[1] FJ767937[1]
Idas sp. Med	Seep	2.129 m	Nile fan (EM)	EF210072[7] FJ159534[1]
I. macdonaldi Gustafson, Turner, Lutz and Vrijenhoek 1998	Seep	650 m	Garden Banks (GoM)	AY649804[1] AY781145[1]

considered as Evolutionary Significant Units (ESUs; Moritz, 1994). As the branching times among ESUs exceed those within ESUs, these entities should also stand up to a criterion of similarity.

In practice, all COI mtDNA sequences obtained from specimens newly sampled from organic falls were pooled with all those available from Genbank (Table 1). The dataset #1 was constituted by adding to these data one sequence from the species *M. modiolus*, which was used as the outgroup following Samadi et al. (2007), and one sequence from each species of a representative subset of all lineages known from hydrothermal vents and cold seeps (Table 2) (Fujita et al., 2009). Using MrModeltest 2.2 (Nylander, 2004) allowed selecting a Generalized Time Reversible (GTR) model, with a gamma law (Γ, four categories) and a proportion of invariants (I), as the best-fitting model of nucleotide evolution. Bayesian trees were calculated under this model with Beast 1.4.8 (Drummond and Rambaut, 2007). A coalescent model of constant population size was used as a tree prior and the heterogeneity of the mutation rate across lineages was set under an uncorrelated log-normal clock. The mutation rate was set to one to get branch lengths in units of substitution per site. Four independent analyses, starting from distinct coalescent trees, were run over 200 million generations and sampled each 10,000 steps. To assess if marginal posterior distributions of each parameter were properly sampled and if the independent runs converged, results were analyzed with Tracer v1.4.1 (Rambaut and Drummond, 2007). After discarding 25 millions samples as a burn-in, the four runs were pooled and resampled each 40,000 steps. The maximum clade credibility tree was drawn from these pooled results. Its branch lengths were conserved and the posterior probabilities of its nodes were calculated from the rest of trees. The resulting tree served as the input in the GMYC model, which was run from the R package (http://www.r-project.org/) using the SPLITS and Ape libraries (Ezard et al. 2009). Kimura 2-parameters (K2P) genetic distances within and among each ESUs defined from the GMYC analysis were estimated using the Mega 4 software and compared to literature data. A maximum likelihood (ML) tree was also build from the dataset #1 using RAxML (Stamatakis, 2006). The best-scoring ML tree under the GTR + Γ + I model was estimated by an analysis starting from a Neighbor-Joining tree built from K2P genetic distances with Mega 4. Robustness of nodes in the best-scoring tree was assessed using the rapid bootstrapping algorithm (1000 replicates) (Felsenstein, 1985; Stamatakis et al., 2008).

The 28S rRNA (unlinked to the COI mtDNA) dataset #2 was built using one sequence from each selected vent and seep species and all sequences available from mussels associated with organic falls. Sequences obtained from mussels associated with organic falls covered all mitochondrial ESUs. GTR + Γ + I with four Γ categories was selected as the best-fitting model of nucleotide evolution. Since the variability of this gene was much lower than that of the COI mtDNA, the GMYC method was not used. Instead, diagnostic alleles were used as a similarity criterion and the monophyly of genetic entities was evaluated from an ML analysis similar to that performed for the dataset #1.

Then, shells of specimens from each ESU were examined to find out shell characters potentially diagnostic. Only high quality pictures from type specimens were available for *A. pacifica*, *I. washingtonia*, *Be. lignocola*, and *Be. geikotsucola*. This examination focused on the general shell shape, the shell thickness and coloration and

the presence/absence of periostracal hairs. Finally, geographical distributions, depth ranges and associations to substrates were also examined to highlight ecological and biogeographical factors that could explain the patterns of genetic and morphologic polymorphism from which the species hypotheses were drawn.

2.4. Phylogenetic relationships

The genetic entities that we identified were then used to reassess the phylogenetic relationships among mussels from organic falls and those from vents and seeps. Only one specimen from each genetic entity was selected from datasets #1 and #2 to build datasets #3 and #4, respectively. Lengths of datasets, alignments and substitution models were the same than those used in the alpha-taxonomy approach. A combined dataset #5 was also built. For this, substitution models were set according to the data partition and mutation rates were relaxed between genes. Both ML and Bayesian approaches were used.

Best-scoring ML trees were estimated using RAxML from 100 independent searches that started from distinct random trees and the robustness of their nodes was assessed using the thorough bootstrapping algorithm (1000 replicates). Bayesian trees were calculated with Beast 1.4.8. The Yule model was used as the tree prior and the heterogeneity of the mutation rate across lineages was set under an uncorrelated log-normal clock. The mean mutation rate was set to one. Four independent analyses, starting from distinct coalescent trees, were run over 10 million and 20 million generations for single and combined datasets, respectively and sampled each 1000 steps. After analyzing results with Tracer v1.4.1 and discarding 50% samples as a burn-in, independent runs were pooled and resampled each 4000 steps. Trees and posterior probabilities of nodes were calculated following the method used for the dataset #1.

2.5. Evolution of habitat use

The evolution of habitat use was assessed using the maximum likelihood method implemented in Mesquite (Maddison and Maddison, 2009). Following the method of Jones et al. (2006), habitat was coded by three possible states, namely "hydrothermal vent", "cold seep" and "organic falls". Neither intra-specific polymorphisms nor various kinds of organic substrates were coded. The evolution of habitat use was set under a Markov model with four states and one transition parameter (Lewis, 2001). To take the tree uncertainties into account, ancestral states were reconstructed for all Bayesian trees retained from the analysis of the dataset #5 and their mean likelihood was then plotted on the maximum clade credibility tree.

3. Results

3.1. Integrative alpha-taxonomy

3.1.1. COI dataset

The COI mtDNA dataset #1 included 390 sequences and 579 positions, of which 269 bp were variable and 238 parsimony informative. Effective Sample Sizes (ESS) of the Bayesian analysis were higher than 300 for all parameters and most were higher than 600, indicating that posterior distributions were well explored. Considering mussels from organic falls, the GMYC method allowed definition of 10 ESUs among previously published data (labeled following the original publications) and two unique haplotypes corresponding to *Be. lignocola* and *I. washingtonia*. Among newly sequenced specimens, 16 ESUs and 1 unique haplotype (labeled from E to U) were defined. For two of these new ESUs, a species name could be attributed. Indeed, the ESU U included only paratypes of *A. arcuatilis*, and among the specimens included in the ESU H there were two sequences of *I. japonica* from Genbank. All ESUs defined were supported by high bootstrap values and/or posterior probabilities, except the ESU F (Fig. 2).

Mean K2P genetic distances within each of the 26 ESUs ranged from 0.1% to 1.8% (Table 3). These values are close although slightly higher than mean intra-specific distances in closely related mussels from hydrothermal vents and cold seeps (~1%, see Miyazaki et al., 2004; Won et al., 2003b). Genetic distances among ESUs ranged from 4.5% to 29% (Supplementary data 3). They therefore exceed the 4% mean divergence between *Ba. thermophilus* and *Ba.* aff *thermophilus*, which represent one of the most recent speciation event clearly identified in vents mussels (Won et al., 2003b).

The ESUs B (*A. crypta*), A (*A. iwaotakii*) and S each included two haplotypic clusters (not shown). B′ and B″ differed by a mean K2P genetic distance of 2.7%, A′ and A″ by 1.7% and S′ and S″ by 2.3% (Table 3). These values are close to those observed in species complexes of mussels from cold seeps (Olu-Le Roy et al., 2007). Only B′, A″ and S′ were monophyletic and rooted within their ESU. The same results were already obtained in our previous study for ESU A and B (Lorion et al., 2009).

3.1.2. 28S dataset

The 28S rRNA gene fragment was successfully sequenced for a subset of 120 additional specimens. Except for *Be. geikotsucola* and *A. pacifica*, ribosomal sequences were therefore available from all mitochondrial ESUs and species from organic falls reported in literature data (Supplementary data 4). After adding *M. modiolus*, and vent/seep species, the dataset #2 included 202 sequences. The alignment had a total length of 1042 positions but the positions 387–418, 426–430, and 690–696 were removed from the dataset because of uncertainties regarding their positional homology. Finally, the dataset #2 was analyzed for 998 positions, of which 174 were variable and 151 were parsimony informative.

Of the 27 mitochondrial lineages from which ribosomal sequences were available, 18 displayed single diagnostic 28S rRNA alleles. Within five ESUs a slight polymorphism was detected (two alleles differing by one or two positions within ESUs A, E, J, Q and P) but alleles were nevertheless diagnostic. In contrast, one allele was shared between ESUs within pairs C/D and H/I. The two minor haplotypic clusters detected within ESU B (*A. crypta*) and within ESU S shared the same single 28S rRNA allele whereas each of the two clusters within ESU A (*A. iwaotakii*) displayed a single diagnostic allele.

Several ribosomal sequences were available for most of the 23 mitochondrial ESUs displaying diagnostic alleles, except *Be. lignocola*, *I. washingtonia* and the specimen M. Most of them were monophyletic, except *Idas* sp. SAL3 and *A. crypta* (ESU B), which were paraphyletic (Supplementary data 4). ESUs within pairs C/D and H/I were mixed with one another, as a consequence of their shared alleles. It is worth noting that the ESU F was here highly supported contrary to the result obtained from the COI mtDNA-based trees.

3.1.3. Morphology

Three morphogroups I, II and III were distinguished (Fig. 2). Within morphogroup I, shells were large (often larger than 1 cm and up to 4 cm), thick, brownish to blackish, and generally without periostracal hairs. High levels of shell polymorphism could be observed in *A. longissima*, ESU E and F, thanks to the many specimens available. The polymorphism was mainly related to the size in *A. longissima*, while both ESU E and F displayed at all sizes continua from straight morphs to curved morphs. While considering the largest specimens – presumably adults – all named species included in the morphogroup I were distinguishable from one

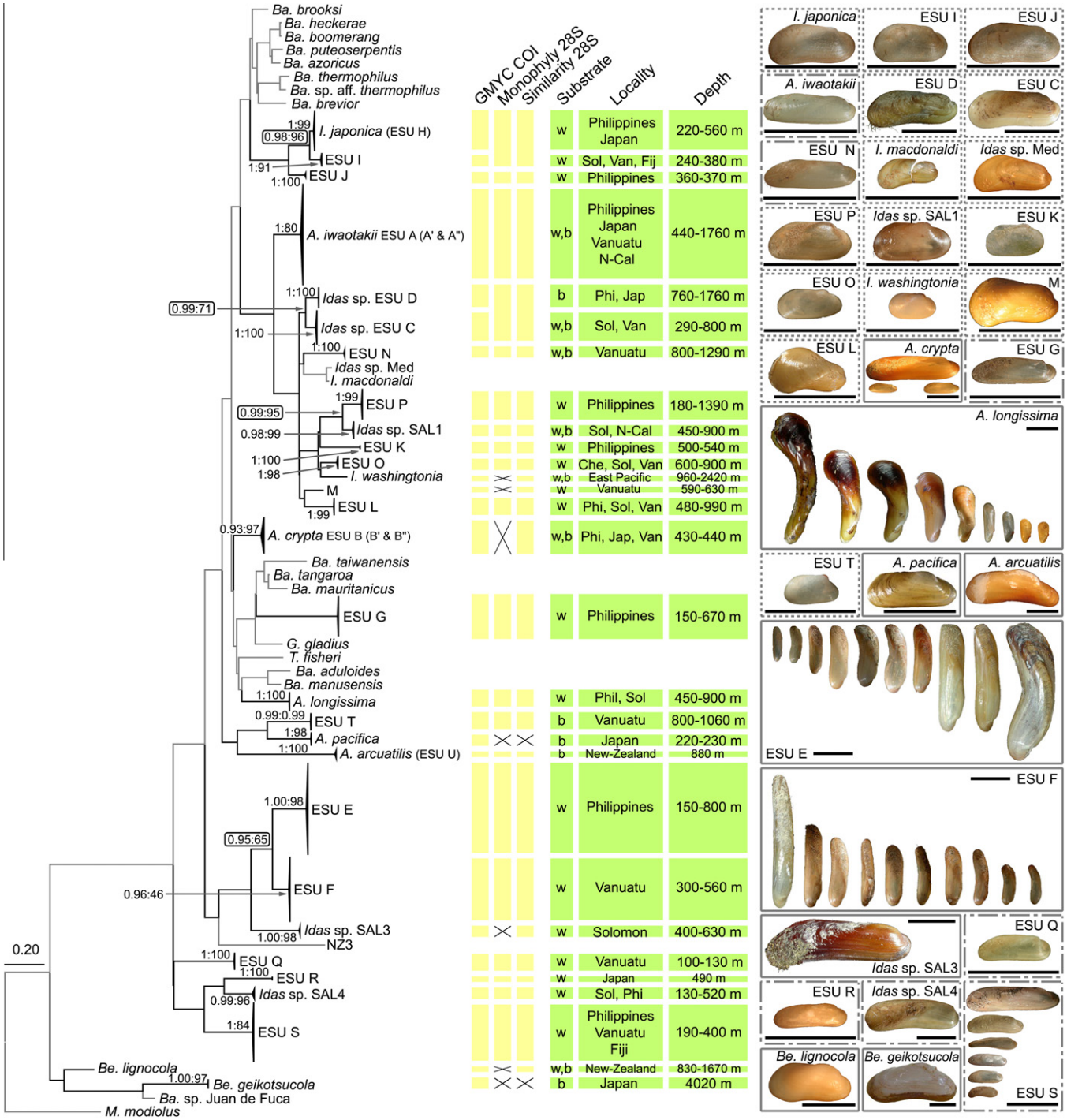

Fig. 2. On the left: Bayesian tree obtained from the analysis of the COI mtDNA (dataset #1). Gray branches correspond to the topology backbone and black branches correspond to lineages from organic falls. Nodes are collapsed at the level of ESUs defined from the GMYC model. For legibility, posterior probabilities and bootstrap supports (from the corresponding ML tree) are only given for ESUs and pairs of ESUs allopatrically distributed (within boxes). The scale bar represents 20% estimated base substitution. On the middle: summary of species hypotheses drawn from the three criterion used and corresponding organic substrate (b: bone, w: wood), localities and depth range. Missing data and paraphyly are marked by black crosses. Localities are sometimes abbreviated by their three first letters. On the right: pictures from specimens representative of each ESU, surrounded by solid, dotted and dashed lines for morphogroups I, II and III, respectively. Black scale bars represent 1 cm length.

another by their shell shape. Largest specimens attributed to *Idas* sp. SAL3 were similar to those attributed to *A. crypta* (ESU B) but differed by having the anterior part of their ventral margin slightly concave. Moreover, shell of the smallest specimens of *Idas* sp. SAL3 were thick, with concave ventral margins and a dark periostracum, whereas they were thinner, convex and more lighter-colored in *A. crypta* (ESU B). The shell polymorphism shared by ESU E and ESU F prevented their diagnosis, but allowed us to distinguish both of them from other species/ESUs within morphogroup I.

Within morphogroup II, shells were rhomboidal to oval, small-sized (from a few millimeters up to 1.5 cm), fragile, yellowish and often with periostracal hairs. No shell polymorphism was detected within any of these ESUs/species, but many included few specimens. ESUs C and D were not distinguished from one another but both differed from other species/ESUs of morphogroup II by

Table 3
Diversity indexes across species, ESUs and haplotypic clusters associated with sunken organic substrates. The number of stations (Nst) and the number of specimens (Nsp) analysed, the number of haplotypes (Hap), and the mean, minimal and maximal K2P genetic distances among haplotypes are given.

Species/ESU	Nst	Nsp	Hap	mean K2P	min K2P	max K2P
Be. lignicola	1	1	1	NA	NA	NA
Be. geikotsucola	1	5	2	0.001	0	0.002
A. pacifica	1	7	4	0.003	0	0.007
A. arcuatilis (ESU U)	1	4	4	0.009	0.003	0.016
I. washingtonia	1	1	1	NA	NA	NA
Idas sp. SAL1	8	9	7	0.011	0	0.030
Idas sp. SAL3	3	4	3	0.005	0	0.007
Idas sp. SAL4	5	5	4	0.012	0.002	0.023
A. longissima	8	10	3	0.001	0	0.004
A. iwaotakii ESU A (A′)	3	50	21	0.002	0	0.009
A. iwaotakii ESU A (A″)	2	3	1	0	0	0
A′ + A″	5	53	22	0.004	0	0.021
A. crypta ESU B (B′)	2	5	4	0.002	0	0.003
A. crypta ESU B (B″)	3	14	7	0.009	0	0.024
B′ + B″	4	19	11	0.018	0	0.036
Idas sp. ESU C	7	18	6	0.002	0	0.009
Idas sp. ESU D	4	11	3	0.001	0	0.004
ESU E	11	49	28	0.004	0	0.018
ESU F	5	34	12	0.004	0	0.025
ESU G	7	21	10	0.003	0	0.007
ESU H	8	19	4	0.001	0	0.005
ESU I	4	7	5	0.004	0	0.009
ESU J	1	2	2	0.005	NA	NA
ESU K	1	2	2	0.002	NA	NA
ESU L	7	9	4	0.003	0	0.010
M	1	1	1	NA	NA	NA
ESU N	2	3	3	0.005	0.002	0.008
ESU O	4	7	4	0.005	0	0.016
ESU P	7	16	11	0.005	0	0.014
ESU Q	2	9	6	0.003	0	0.007
ESU R	1	2	2	0.005	NA	NA
I. japonica ESU S (S′)	4	5	4	0.003	0	0.007
I. japonica ESUS (S″)	5	26	12	0.005	0	0.010
S′ + S″	9	31	16	0.01	0	0.028
T	2	9	1	0	0	0

having a concave ventral margin. ESUs L and M differed from other species/ESUs of morphogroup II by displaying a strong angle between dorsal and ventral margins. Such angles between dorsal and ventral margins are yet reported in *Idas* sp. Med and *I. macdonaldi* but much less marked (Gustafson et al., 1998; pers. obs.). In ESU L, the angle started from the umbo which was terminal, while in M, the angle started at the midpoint of the dorsal margin and the umbo was subterminal. The ventral and dorsal margins were straight and almost parallel in other species/ESUs and no consistent morphological difference was found among them.

Shells within morphogroup III were elongated, fragile, small-sized (from a few millimeters up to 1.5 cm), yellowish, and often with periostracal hair. A high level of shell polymorphism was observed within ESU S, whose anterior parts of the shells were more or less thin. Other species/ESUs did not display any shell polymorphism, but few specimens were available for many of them. No consistent morphological difference was found among species from morphogroup III.

3.1.4. Bathymetry and association to substrate

ESUs/species as defined herein were collected from 100 m to 1.400 m depth (Fig. 2). So far there is no evidence that species from organic falls can live in shallower water. While equipment used during the Tropical Deep-Sea Benthos cruises prevented deeper sampling, a few species reported in the literature were collected up to 4000 m depth (Table 3). It is worth noting that most ESUs including specimens from many stations were collected over large depth ranges. Narrow depth ranges sometimes observed might therefore reflect sampling biases.

Most ESUs were collected only from sunken wood while ESU T and *A. arcuatilis* (ESU U) were collected only from turtle and whale bone, respectively. This study also allowed identification of new ESUs able to colonize both sunken wood and bone in addition to ESUs C and B already reported by Lorion et al. (2009). Indeed, specimens of *A. iwaotakii* (ESU A) and *Idas* sp. SAL1 were collected from turtle bone whereas these species had been previously only sampled from sunken wood (Samadi et al., 2007). Similarly, the new ESU N was also collected on both sunken wood and turtle bone.

3.1.5. Geographical patterns

Most species/ESUs collected in archipelagos from the North-Western part of the sampling plan (Japan and Philippines) were not recovered in archipelagos from the South-Eastern part (Solomon Islands, Vanuatu, Chesterfields, New Caledonia, Fiji and New-Zealand) (Fig. 2). Additional levels of geographic structure were detected off the Philippines, where the species distribution did not overlap between the Bohol Sea and the Philippines Sea, and off Vanuatu, where only 2 of the 12 ESUs/species were collected in both Big Bay and Southern localities (Supplementary data 2).

This geographic distribution was highlighted by the phylogenetic analyses performed on the COI mtDNA dataset #1 (Fig. 2). Indeed, four pairs of ESUs were distributed on both sides of the equator. For example, ESU P collected off the Philippines was sister group of *Idas* sp. SAL1 from the Solomon Islands. This geographic pattern of genetic structure was also found within ESUs A, B and S and explained haplotypic clusters detected in them. These results confirmed and added support to our previous study, in which the geographic pattern between ESUs C and D and within ESUs A and B was already found (Lorion et al. 2009). Of these seven pairs of haplotypic clusters distributed on both sides of the equator, three (A′/A″, ESU E/ESU F, *Idas* sp. SAL1/ESU P) were recovered from the analysis of the 28S rRNA, along with a new one between ESUs O and K (Supplementary data 3).

3.2. Phylogenetics relationships and evolution of habitat use

In the COI mtDNA dataset #3, 242 base pairs were variable and 224 parsimony informative. In the 28S rRNA dataset #4, 160 base pairs were variable and 92 bp parsimony informative. ESS were higher than 1000 in all Bayesian analyses. ML and Bayesian analyses of datasets #3 and #4 were consistent with ML trees obtained from analyses of the datasets #1 and #2, respectively (Supplementary data 5 and 6), and consistent with one another. Analyses of the combined dataset #5 allowed us to resolve a few additional nodes (Fig. 3).

Consistent with previous studies *Be. lignocola* appeared as the sister species of all other lineages that were included in a well-supported clade. This clade included seven lineages supported by bootstrap values and posterior probabilities higher than 80% and equal to 1, respectively, and seven single species or species complexes. The two main lineages from hydrothermal vents and cold seeps, namely the "*thermophilus*" (L5), "*childressi*" (L10) lineages, were recovered as in the study by Jones et al. (2006). We could moreover confirm the relationship of the new species *Ba. taiwanensis* with the "*childressi*" group, as previously proposed in an anatomical study (Cosel, 2008). Among the new lineages recovered by our analysis, L6 was the most highly diversified among deep-sea mussels. This lineage included mostly mussels from organic falls but also *I. macdonaldi* and *Idas* sp. Med that live at cold seeps. The remaining lineages including several species were associated with organic falls.

Although the relationships among lineages were generally poorly resolved, a sister group relationship was displayed between

(i) the lineage L6 and the "*thermophilus*" lineage (L5) and (ii) the sunken-wood lineage L2 and the vent species NZ3 (L3). Habitat transitions that could be expected from these results were confirmed by the maximum likelihood analysis of the evolution of habitat use. This reconstruction of ancestral states moreover suggested that organic falls were the ancestral substrate of all deep-sea mussels, although the alternative hypothesis could not be rejected at a 5% threshold (Fig. 4).

The lineages associated with organic falls generally included mussels belonging to the same morphogroup (Fig. 3). However, each of the three morphogroups was split into several lineages. This situation may result from the lack of resolution at deep nodes. However, a lack of resolution explained this result only partly since some robustly supported lineages included several morphogroups (e.g. all mussels of L6 belonged to morphogroup II, except ESU N and *A. iwaotakii*).

4. Discussion

4.1. Species delimitation: integrating various lines of evidence

Our first aim was to reassess the alpha-diversity of mussels associated with sunken organic substrates from an extensive sampling in the South-West Pacific. To propose primary hypotheses of species delimitations from our mitochondrial dataset, we used the GMYC model that aims to distinguish coalescent from speciation branching patterns. Overall, it allowed us to detect 26 ESUs and three unique haplotypes. Of these 29 lineages, 17 were new to science. Phylogenetic trees revealed that all mitochondrial ESUs, but one, were highly supported by bootstrap values and posterior probabilities. These ESUs therefore stand up to a phylogenetic criterion for species delimitation. They also stand up to a similarity criterion, as mean K2P genetic distances within and among ESUs

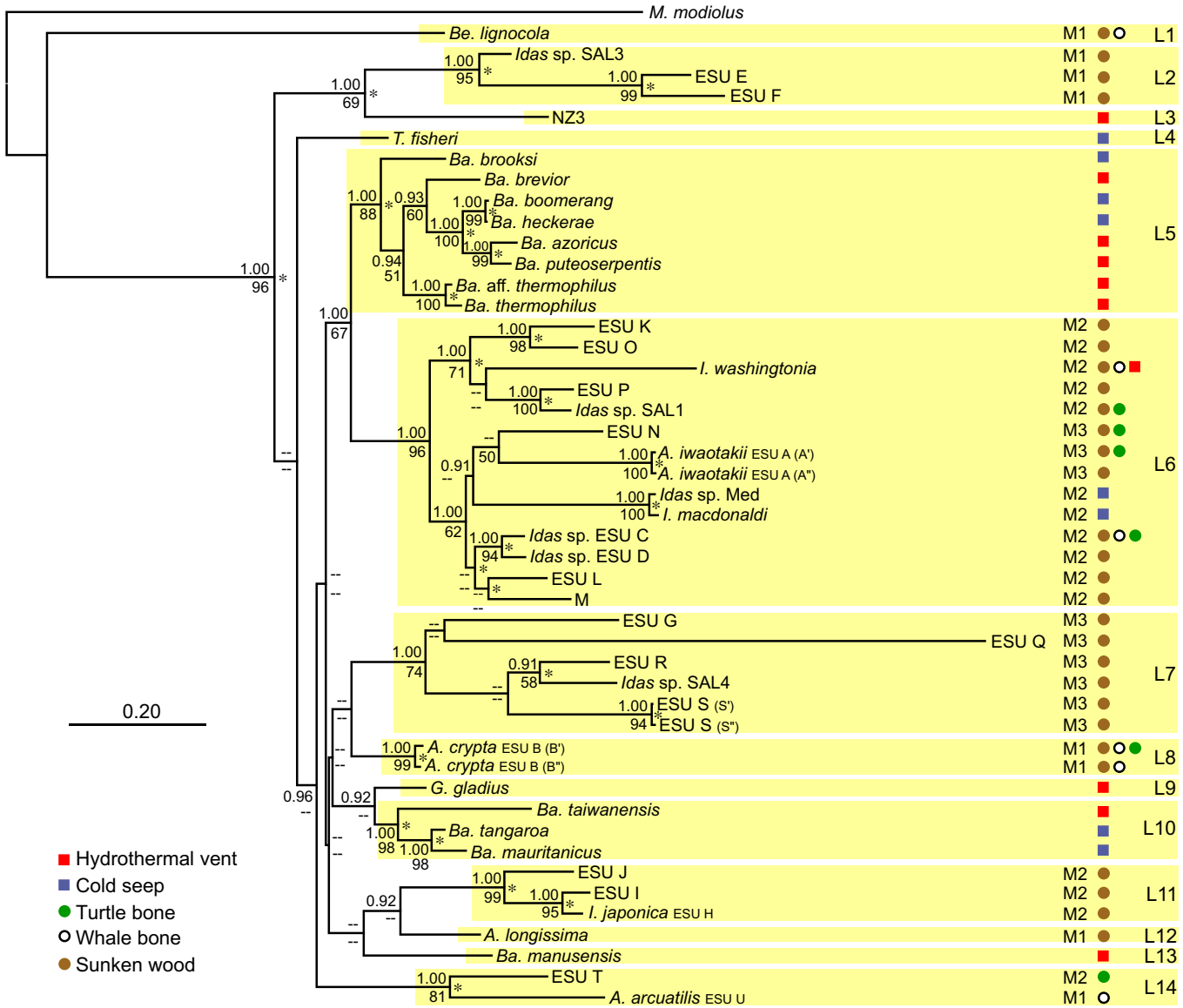

Fig. 3. Maximum clade credibility phylogram obtained from Bayesian analyses of the combined dataset #5, including species from hydrothermal vents, cold seeps and organic falls obtained from Genbank and new samples. Values above and below nodes correspond to posterior probabilities (PP) and bootstrap values (BP) obtained from Bayesian and Maximum Likelihood analyses, respectively. PP below 0.90 and BP below 50 are not shown. Nodes recovered independently from the two genes trees (Supplementary data 5 and 6) are marked with stars. Labels L1 to L14 correspond to lineages discussed in the text. Environments inhabited by each species and morphogroups (M1, M2 and M3) of species from organic falls are given. The scale bar represents to 20% estimated base substitution.

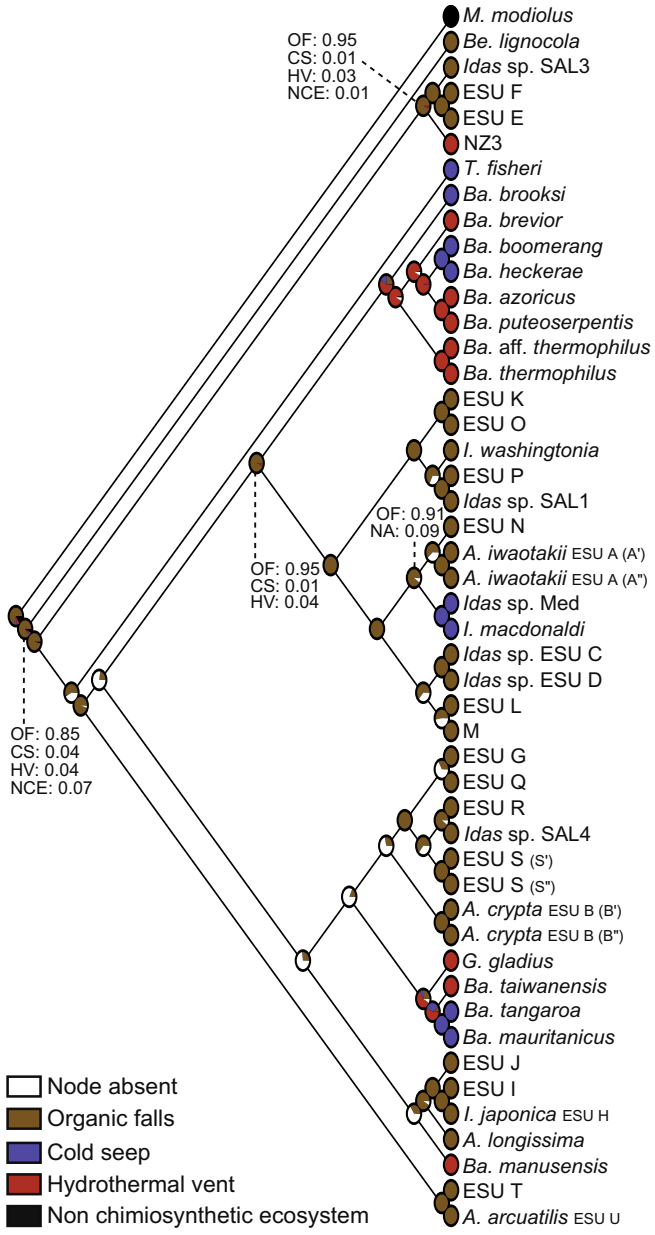

Fig. 4. Maximum likelihood reconstruction of ancestral habitats. Pie charts correspond to average likelihoods of each state, which were plotted on the maximum clade credibility tree obtained from Bayesian analyses of the combined dataset #5. Numeric values at nodes discussed in the text are given (HV: hydrothermal vent, CS: cold seep, OF: organic falls, NCE: non chimiosynthetic ecosystem, NA: node absent).

were similar to intra-specific and inter-specific genetic distances, respectively, reported for vent and seep mussels.

Ribosomal sequences were available for 27 of the 29 mitochondrial ESUs. Among them, 18 displayed diagnostic alleles and were monophyletic from the 28S rRNA-based tree. Despite that several ribosomal sequences were obtained from these ESUs, and that they co-occurred at several stations, there was no evidence of introgression. These 18 ESUs may therefore also stand up to a criterion of reproductive isolation and constitute among the most robust species hypotheses that can be drawn from our molecular dataset.

In some cases, similarity and phylogenetic criteria could not be simultaneously validated because (i) no data were available for the 28S gene fragment (*A. pacifica* and *Be. geikotsucola*), (ii) the mitochondrial ESUs were paraphyletic in the 28S rRNA-based tree (*Idas* sp. SAL3, *A. crypta* ESU B), and (iii) data were available only for one specimen (*Be. lignocola*, *I. washingtonia* and M). However, in these seven cases, COI mtDNA-based hypotheses seems robust with respect to either monophyly on this gene or genetic distances from other ESUs for both genes. Moreover, most of them except *I. washingtonia* corresponded to morphologically diagnosable entities, which provides additional support for their species status.

Some cases are more ambiguous. The mitochondrial divergence between ESU C and D and between ESU H and I was high (8.9% and 4.8% respectively), but each shared with its close relative a slight amount of nuclear polymorphism. Moreover ESUs within each pair were morphologically indistinguishable from one another. Such genetic patterns might be explained either by an incomplete lineage sorting or by introgression. An incomplete lineage sorting of the 28S rRNA seems *a priori* the most probable explanation (Nichols, 2001). However, gene flows were reported within the "*childressi*" complex and between *Ba. azoricus* and *Ba. puteoserpentis* despite mean levels of mitochondrial divergence of 3% and 7.4%, respectively (Won et al., 2003a; Olu-Le Roy et al., 2007). Such introgression patterns are common in Mytilidae and thus cannot be ruled out without analyzing additional data (Bierne et al., 2003).

Within three ESUs additional levels of genetic structure were detected. Indeed, ESU S, *A. iwaotakii* (ESU A) and *A. crypta* (ESU B) may each be split into two distinct mitochondrial clusters. However, these lineages were not reciprocally monophyletic. Moreover each pair, except *A. iwaotakii* (ESUA), shared a single 28S rRNA allele. As haplotypic clusters within each pair did not geographically overlap, this pattern could be explained by a marked geographic structure within species. However, the hypothesis of a lack of differentiation between recently separate species cannot be ruled out. The ESU S, *A. crypta* (ESU B) and *A. iwaotakii* (ESU A) might therefore be referred to as species complexes until more data are available.

Overall our results highlight a molecular diversity that was unexpected given the taxonomic literature and our preliminary morphological observations. This study should be considered as a starting point for further investigations of the diversity in mussels associated with organic falls in the Western Pacific. Indeed, the diversity structure at both large and regional scales suggests that sampling new archipelagos and new localities within archipelagos, respectively, will likely allow the discovery of more species. This and population genetics are also required to refine some of the hypotheses presented here.

4.2. Speciation patterns in mussels from organic falls

Phylogenetic trees presented herein allowed the distinction of several new well-supported lineages of mussels mainly associated with organic falls. Moreover, we detected a recurrent geographic pattern of genetic structure within pairs of closely related mussels. Indeed, for five pairs of ESUs, the two sister-ESUs had no overlapping distributions in our dataset. For each of these five pairs, one ESU was located in the north-western part of our sampling plan (Japan and the Philippines) and the second one in the South-Eastern part (Solomon Islands, Vanuatu, Chesterfields, Fiji and New Zealand). The same geographic pattern was discovered between haplotypic clusters depicted within three other ESUs.

The oldest paleontological records of species associated with organic falls and species from vent and seeps date both from late Eocene (Amano et al., 2007; Kiel and Goedert, 2006b, 2007; Kiel et al., 2010). At this time, the whole Indo-West Pacific area was undergoing intense paleo-oceanographic re-arrangements, as consequences of the collision of both Australia and New Guinea with the South-East Asia (Hall, 1998, 2001, 2002). These paleotectonic events allowed the creation of vast amounts of new shallow waters, habitat fragmentation, and subsequent diversification of

numerous marine taxa 20–30 Myr ago (Crame and Rosen, 2002; Meyer et al., 2005; Williams, 2007; Williams et al., 2008). Rising islands likely increased the number of areas where plant remains could accumulate and mussel thrive. Therefore it may explain at least partly the diversity observed in our data set.

However deep-sea mussels have bentho-pelagic life cycles that include highly dispersive larvae (Dean, 1993; Lutz et al., 1984). These larvae may be able to spend up to six months in the water column, which could explain low levels of spatial genetic structure over hundreds and even thousands of kilometers (Olu-Le Roy et al., 2007; Won et al., 2003b). We showed that several species are able to colonize not only wood but also whale and turtle bones, the distribution of which is not related to proximity to land (Lorion et al., 2009; this study). Furthermore, islands such as the Halmahera Island, Papua New Guinea and its associated arcs, occurred between the Philippines and the Melanesian arc during the last 40 Myr (Hall, 2001). This suggests that many unevenly distributed patches of organic matter (e.g. our samples from the Coral Sea, collected far from any coast) should have constituted effective stepping stones among archipelagos and prevented any divergence process.

Therefore the geographic patterns of genetic structure found in this study cannot be explained without assuming the occurrence of barriers to dispersal. Interestingly, there are many marine organisms that display geographical patterns consistent with the split between North-Western/South Eastern localities observed in our dataset (Macaranas et al., 1992; McMillan and Palumbi, 1995; Palumbi, 1997; Benzie, 1998; Planes and Fauvelot, 2002; Barber et al., 2006; Imron et al., 2007; Magsino and Juinio-Meñez, 2008). These patterns are suggested to result from (i) the South Philippines split of the Equatorial Counter Current into the northward-flowing Kuroshio and the southward-flowing current Mindanao, and/or (ii) Pleistocene sea levels changes (see the review by Hoeksema, 2007). The eustatic variations within the last 40 Myr (Miller et al., 2005; Haq and Schutter, 2008) should also have conditioned the number of wood patches surrounding islands and thus, the habitat connectivity at organic falls.

Since the mussels studied here have similar depth ranges and are all thought to be highly dispersive, they should have been affected by eustatic changes and ocean circulation in a similar way. However, the consistency of geographical patterns observed herein contrasts with the heterogeneity of branch lengths among pairs of ESUs/clusters (Fig. 3). One may hypothesize that subtle differences in pelagic larval durations may have influenced the speed of the divergence processes. This hypothesis is sound as both lecitotrophic and planktotrophic larvae are reported in mussels from organic falls (Horikoshi and Tsuchida, 1984; Dean, 1993). Other factors, such as differences in mutation rates, effective population sizes and selective constraints may also explain this heterogeneity.

Given the large spatial and temporal scales we considered, the diversification processes explaining the geographical patterns presented here likely involve not only drastic Cenozoic paleo-oceanographic re-arrangements but also later eustatic changes and ocean circulation. The interaction among physical factors and heterogeneous biological features may have even more increased the complexity of resulting patterns, as suggested elsewhere (Paulay, 1997; Meyer et al., 2005).

5.3. New insights to the "wooden-step" hypothesis

The "wooden-step" hypothesis, formulated by Distel et al. (2000), suggests that mussels from hydrothermal vents and cold seeps arose from a recent adaptation of mussels associated with organic falls. The multiplicity of colonization events toward vents and seeps was first suggested by Jones et al. (2006). Indeed, they highlighted that the vent species NZ3 is a basal lineage distinct from other vent and seep mussels. These, namely the "*thermophilus*" lineage, the "*childressi*" lineage and *T. fisheri*, clustered together. Their results were not challenged by other studies that also included a fourth vent lineage, including the species *Ba. manusensis* and *Ba. aduloides* (Iwasaki et al., 2006; Won et al., 2008). However, all these studies have sampled with a strong bias towards vent and seep species.

Correcting this sampling bias first allowed us to suggest a sister group relationship between the "*thermophilus*" lineage and a lineage including only *Idas* and *Adipicola* mussels (L6). Second, we showed that the paraphyletic assemblage of NZ3 and a sunken-wood mussels at the root of the tree in our previous study (Samadi et al., 2007) is in fact monophyletic. The addition of new species belonging to those lineages may have prevented long-branch effects and these new results, recovered independently from the two gene trees, are *a priori* more robust. Third, we found a lineage (L6) that included species from organic falls along with *Idas* sp. Med and *I. macdonaldi*, reported from cold seeps, and *I. washingtonia*, reported from both hydrothermal vents and organic falls (Dell, 1987; Gustafson et al., 1998; Southward, 2008). Overall, this study provides new lines of evidence that vent and seep mussels arose from multiple colonization events and that their history is closely linked to that of species associated with organic falls. Cohesively with our results, a recent analysis of the COI and ND4 mtDNA encoding-genes (Kyuno et al., 2009; see also Fujita et al., 2009) showed a close relationship between *A. crypta*, associated with organic falls, and the "*childressi*" group, living at vents and seeps only. It is worth noting the diversity of habitats used by species/ESUs within lineage 6. Indeed, this suggests that these mussels are less specialized with regard to the environment than other vent and seep species attributed to the polyphyletic Bathymodiolinae and is thus in accordance with expectations of the "wooden-step" hypothesis.

It is suggested that the radiation of deep-sea mussels resulted from an adaptive process from shallow seeps to deep vents (Jones et al., 2006). This hypothesis is however substantially challenged by the broad depth range of many species and lineages (Kyuno et al., 2009; this study). The symbiotic relationships may have played a role in the diversification process by increasing the metabolic capabilities, and thereby the number of ecological niches that mussels could colonize (Cavanaugh et al., 2006). Habitat heterogeneity (such as differences in the chemical composition of fluids) may have driven diversification through associations to symbionts having different metabolic capabilities. However, many, if not most, deep-sea mussels have only thiotrophic symbionts (Duperron et al., 2009), whose genetic structure reflects geography rather than ecology (Won et al., 2008; Lorion et al., 2009). Although the symbiotic relationships were a pre-requisite to the colonization of chemosynthetic ecosystems by mussels, their exclusive role in the subsequent diversification is therefore questionable. This diversification might also be ascribed to many other physiological adaptations. As an alternative to adaptive hypotheses, divergence processes in mussels were also shown to be driven by geography (Won et al., 2003b; Olu-Le Roy et al., 2007; Faure et al., 2009; Plouviez et al., 2009). By putting once again the role of the geography in mussel speciation forward, the present study further qualifies the adaptive scenarios previously proposed to explain the diversification of deep-sea mussels.

4.4. The shell handicap of mytilid systematics

As already reported in deep-sea mussels (Won et al., 2003b) and other Mytilidae (Lee and Foighil, 2004), many species identified in our molecular analyses have similar looking shells and may be morphologically cryptic. In other cases, the shell polymorphism was higher within species than between them, thus often preventing objective morphological diagnosis. These levels of polymorphism

may reflect either allometric growth, as shown in the wood-associated species *A. longissima* (Horikoshi and Tsuchida, 1984), or environmental plasticity, a phenomenon that is very common in molluscs (Baker et al., 2003; Hollander et al., 2006; Wullschleger and Jokela, 2002; Yeap et al., 2001). Mussels sampled on sunken-woods are found on the outer surface or within small cavities. In this case, the shell shape seems to be constrained by the shape of cavities (pers. obs.).

The poor evolutionary significance of shell shapes along with the few characters used in most descriptions of species from organic falls did not enable us to link all species to extant names. In this context, only species that were very easy to identify (*A. longissima* and *A. crypta*) or those that included type specimens (*I. macdonaldi* and *A. arcuatilis*) could be attributed to a name with confidence. Within morphogroups II and III, species identifications that do not involve type specimens or type localities (*A. iwaotakii*, *I. japonica* and *I. washingtonia*) may be questioned. As most type of specimens in mussels from organic falls consist of dried and empty shells, there will always be uncertainty regarding the use of names based upon them, the implication being that neotypes will be required.

Our data also confirm the poor relevance of genus descriptions in mussels from organic falls. Because of their relationships with polyphyletic Bathymodiolinae from vents and seeps, species defined herein should be included in the global systematic revision of deep-sea mussels suggested by various authors (Cosel, 2002; Jones et al., 2006; Kyuno et al., 2009). Such a revision should be made on the ground of a robust phylogenetic framework that is not yet fully resolved. Rather than just using shell characters the literature data suggest that anatomical characters would be very valuable to both species and genus delimitation (Cosel, 2002; Dell, 1987; Gustafson et al., 1998). Indeed, the polyphyly of vent and seep Bathymodiolinae was already suggested in such data. Among others, characters related to the mantle and muscle configuration have been used to suggest that the Bathymodiolinae should be split into a "*childressi*" group and a "*thermophilus*" group (Cosel, 2002). Based on these characters, the new species *Ba. taiwanensis* was attributed to the "*childressi*" group (Cosel, 2008), a result here supported with molecular data. These examples (see also Dell, 1987) are encouraging clues for forthcoming anatomical studies in mussels associated with organic falls.

5. Conclusion

We used an explorative and integrative approach to taxonomy to overcome problems related to the taxonomy of mussels from organic falls. The comprehensive taxonomic framework we built allowed us to rectify the bias of previous studies and the relationships of mussels from the three kinds of deep-sea chemosynthetic environments to be better evaluated. The allopatric pattern and the poor specialization of mussels with regard to the substrate, interestingly, challenge adaptive scenarios usually proposed to explain their diversification. A more resolved phylogeny is however still needed to further assess systematics and evolutionary processes in the whole group.

Acknowledgments

This work was supported by the "Consortium National de Recherche en Génomique" and the "Service de Systématique Moléculaire" (UMS 2700 CNRS-MNHN). It is part of the agreement no. 2005/67 between the Genoscope and the Muséum National d'Histoire Naturelle on the project "Macrophylogeny of life" directed by Guillaume Lecointre. The computations were performed at the Vital-IT (http://www.vital-it.ch) Center for high-performance computing of the Swiss Institute of Bioinformatics. New material was collected onboard of M/VDA-BFAR (PANGLAO 2005, Co-PI P. Bouchet and L. Labe and Aurora 2007, Co-PI M. Manuel, and P. Bouchet) and onboard of R/V Alis (SantoBOA and BOA1, Co-PI B. Richer de Forges and S. Samadi; SANTO2006, Co-PI P. Bouchet, O. Pascal and H. Le Guyader; BORDAU 1, Salomon2 and EBISCO, Co-PI B. Richer de Forges and P. Bouchet). The sampling would not have been possible without the following funding agencies: Total Foundation, Philippines Bureau of Fisheries and Aquatic Resources, National Museum of the Philippines, and Lounsbery Foundation. We gratefully acknowledge M-C Boisselier, T. Comtet, R. von Cosel, S. Duperron, B. Faure, Y. Fujiwara, T. Haga, D. Jollivet, S. Kiel, B. Marshall, J.-I. Miyazaki, and S. Tillier, for providing additional samples, field work and highlighting discussions, S. Ho for his kind help with Bayesian analyses, C. Little, B. Marshall and two anonymous reviewers for constructive comments and English improvement of earlier versions of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.05.027.

References

Amano, K., Little, C.T.S., Inoue, K., 2007. A new Miocene whale-fall community from Japan. Palaeogeogr. Palaeoclimatol. Palaeoecol. 247, 236–242.
Baker, A.M., Bartlett, C., Bunn, S.E., Goudkamp, K., Sheldon, F., Hughes, J.M., 2003. Cryptic species and morphological plasticity in long-lived bivalves (Unionoida: Hyriidae) from inland Australia. Mol. Ecol. 12, 2707–2717.
Barber, P.H., Erdmann, M.V., Palumbi, S.R., Ayre, D., 2006. Comparative phylogeography of three codistributed stomatopods: origin and timing of regional lineage diversification in the coral triangle. Evolution 60, 1825–1839.
Benzie, J.A.H., 1998. Genetic structure of marine organisms and SE Asian biogeography. In: Hall, R., Holloway, J.D. (Eds.), Biogeography and Geological Evolution of SE Asia. Backhuis Publishers, Leiden, pp. 197–209.
Bierne, N., Borsa, P., Daguin, C., Jollivet, D., Viard, F., Bonhomme, F., David, P., 2003. Introgression patterns in the mosaic hybrid zone between *Mytilus edulis* and *M. galloprovincialis*. Mol. Ecol. 12, 447–461.
Blaxter, M., 2003. Counting angels with DNA. Nature 421, 122–124.
Blaxter, M.L., 2004. The promise of a DNA taxonomy. Philos. Trans. R. Soc. B 359, 669–679.
Bouchet, P., Heros, V., Lozouet, P., Maestrati, P., 2008. A quater-century of deep-sea malacological exploration in the South and West Pacific: WHERE do we stand? How far to go? Mem. Mus. Natl. Hist. Nat. Ser. A (Paris) 25, 9–40.
Cavanaugh, C., McKiness, Z., Newton, I., Stewart, F., 2006. Marine chemosynthetic symbioses. In: The Prokaryotes. Springer, New York, pp. 475–507. doi:10.1007/0-387-30741-9.
Cosel, R., 2002. A new species of bathymodioline mussel (Mollusca, Bivalvia, Mytilidae) from Mauritania (West Africa), with comments on the genus *Bathymodiolus* Kenk & Wilson, 1985. Zoosystema 24, 259–272.
Cosel, R., 2008. A new bathymodioline mussel (Bivalvia: Mytiloidea: Mytilidae: Bathymodiolinae) from vent sites near Kueishan island, North East Taiwan. Raffles Bull. Zool. 19, 105–114.
Crame, J.A., Rosen, B.R., 2002. Cenozoic palaeogeography and the rise of modern biodiversity patterns. In: Crame, J.A., Owen, A.W. (Eds.), Palaeogeography and Biodiversity Change: The Ordovician and Mesozoic–Cenozoic Radiations. Geological Society, London, pp. 153–168.
De Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56, 879–886.
Dean, H.K., 1993. A population study of the bivalve *Idas argenteus* Jeffreys, 1876, (Bivalvia: Mytilidae) recovered from a submerged wood block in the deep North Atlantic Ocean. Malacologia 35, 21–41.
Dell, R.K., 1987. Mollusca of the family Mytilidae (Bivalvia) associated with organic remains from deep water off New Zealand, with revisions of the genera *Adipicola* Dautzenberg, 1927 and *Idasola* Iredale, 1915. Natl. Mus. New Zealand Rec. 3, 17–36.
Distel, D.L., Baco, A.R., Chuang, E., Morrill, W., Cavanaugh, C.M., Smith, C.R., 2000. Do mussels take wooden steps to deep-sea vents? Nature 403, 725–726.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 1–8.
Dubilier, N., Bergin, C., Lott, C., 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat. Rev. Microbiol. 6, 725–740.
Duperron, S., Halary, S., Lorion, J., Sibuet, M., Gaill, F., 2008a. Unexpected co-occurrence of six bacterial symbionts in the gills of the cold seep mussel *Idas* sp. (Bivalvia: Mytilidae). Environ. Microbiol. 10, 433–445.

Duperron, S., Laurent, M.C.Z., Gaill, F., Gros, O., 2008b. Sulphur-oxidizing extracellular bacteria in the gills of Mytilidae associated with wood falls. FEMS Microbiol. Ecol. 63, 338–349.
Duperron, S., Lorion, J., Samadi, S., Gros, O., Gaill, F., 2009. Symbioses between deep-sea mussels (Mytilidae: Bathymodiolinae) and chemosynthetic bacteria: diversity, function and evolution. C. R. Biol. 332, 298–310.
Ezard, T., Fujisawa, T., Baraclough, T., 2009. SPecies LImits by Threshold Statistics. <http://r-forge.r-project.org/projects/splits/>.
Faure, B., Jollivet, D., Tanguy, A., Bonhomme, F., Bierne, N., 2009. Speciation in the deep sea: multi-locus analysis of divergence and gene flow between two hybridizing species of hydrothermal vent mussels. Plos One 4, e6485. doi:10.1371/journal.pone.0006485.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Folmer, O., Black, M., Hoeh, W.R., Lutz, R.A., Vrijenhoek, R.C., 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Fontaneto, D., Herniou, E.A., Boschetti, C., Caprioli, M., Melone, G., Ricci, C., Barraclough, T.G., 2007. Independently evolving species in asexual bdelloid rotifers. PLoS Biol. 5, e87.
Fujita, Y., Matsumoto, H., Fujiwara, Y., Hashimoto, J., Sergey, V., Galkin, S.V., Ueshima, R., Miyazaki, J.-I., 2009. Phylogenetic relationships of deep-sea *Bathymodiolus* mussels to their mytilid relatives from sunken whale carcasses and wood. Venus 64, 123–134.
Gustafson, R.G., Turner, R.D., Lutz, R.A., Vrijenhoek, R.C., 1998. A new genus and five new species of mussels (Bivalvia, Mytilidae) from deep-sea sulfide/hydrocarbon seeps in the Gulf of Mexico. Malacologia 40, 63–112.
Hall, J., 2001. Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. In: Metcalfe, I., Smith, J.M.B., Morwood, M., Davidson, I.D. (Eds.), Faunal and Floral Migrations and Evolution in SE Asia–Australasia. Swets & Zeitlinger, Lisse, pp. 35–56.
Hall, R., 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall, R., Holloway, J.D. (Eds.), Biogeography and Geological Evolution of SE Asia. Blackhuis Publishers, Leiden, pp. 99–131.
Hall, R., 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. J. Asian Earth Sci. 20, 353–431.
Haq, B.U., Schutter, S.R., 2008. A chronology of Paleozoic sea-level changes. Science 322, 64–68.
Hoeksema, B., 2007. Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle. In: Renema, W. (Ed.), Biogeography, Time, and Place: Distributions, Barriers, and Islands. Springer, Dordrecht, pp. 117–178. doi:10.1007/978-1-4020-6374-9.
Hollander, J., Collyer, M.L., Adams, D.C., Johannesson, K., 2006. Phenotypic plasticity in two marine snails: constraints superseding life history. J. Evol. Biol. 19, 1861–1872.
Horikoshi, M., Tsuchida, E., 1984. Distribution, mode of life and allometric growth of a deep-sea Mytilid bivalve, *Adipicola longissima* (Thiele et Jaeckel, 1931). Venus 43, 86–100.
Hourdez, S., Lallier, F.H., 2006. Adaptations to hypoxia in hydrothermal-vent and cold-seep invertebrates. Rev. Environ. Sci. Bio/Technol. 6, 143–159.
Imron, Jeffrey, B., Hale, P., Degnan, B.M., Degnan, S.M., 2007. Pleistocene isolation and recent gene flow in *Haliotis asinina*, an Indo-Pacific vetigastropod with limited dispersal capacity. Mol. Ecol. 16, 289–304.
Iwasaki, H., Kyuno, A., Shintaku, M., Fujita, Y., Fujiwara, Y., Fujikura, K., Hashimoto, J., Martins, L., Gebruk, A., Miyazaki, J.-I., 2006. Evolutionary relationships of deep-sea mussels inferred by mitochondrial DNA sequences. Mar. Biol. 5, 1–12.
Johnson, S.B., Warén, A., Vrijenhoek, R.C., 2009. DNA barcoding of *Lepetodrilus* limpets reveals cryptic species. J. Shellfish Res. 27, 43–51.
Jones, W.J., Won, Y.-J., Maas, P.A.Y., Smith, P.J., Lutz, R.A., Vrijenhoek, R.C., 2006. Evolution of habitat use by deep-sea mussels. Mar. Biol. 148, 841–851.
Kiel, S., Campbell, K.A., Gaillard, C., 2010. New and little known mollusks from ancient chemosynthetic environments. Zootaxa 2390, 26–48.
Kiel, S., Goedert, J., 2006a. Deep-sea food bonanzas: early Cenozoic whale-fall communities resemble wood-fall rather than seep communities. Proc. R. Soc. Lond. Ser. B 273, 2625–2631.
Kiel, S., Goedert, J.L., 2006b. A wood-fall association from late Eocene deep-water sediments of Washington state, USA. Palaios 21, 548–556.
Kiel, S., Goedert, J.L., 2007. New mollusks associated with biogenic substrates in Cenozoic deep-water sediments of Washington State. Acta Palaeontol. Pol. 52, 41–52.
Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420, 73–90.
Kyuno, A., Shintaku, M., Fujita, Y., Matsumoto, H., Utsumi, M., Wanatabe, H., Fujiwara, Y., Miyazaki, J.-I., 2009. Dispersal, differentiation of deep-sea mussels of the genus Bathymodiolus (Mytilidae, Bathymodiolinae). J. Mar. Biol. 72. doi:10.1155/2009/625672. 15pp. (Article ID 6256).
Lee, T., Foighil, D.O., 2004. Hidden Floridian biodiversity: mitochondrial and nuclear gene trees reveal four cryptic species within the scorched mussel, *Brachidontes exustus*, species complex. Mol. Ecol. 13, 3527–3542.
Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925.
Little, C.T.S., Vrijenhoek, R.C., 2003. Are hydrothermal vent animals living fossils? Trends Ecol. Evol. 18, 582–588.
Lonsdale, P.F., 1977. Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. Deep-Sea Res. Part II 24, 857–858.
Lorion, J., Duperron, S., Gros, O., Cruaud, C., Samadi, S., 2009. Several deep-sea mussels and their associated symbionts are able to live both on wood and on whale falls. Proc. R. Soc. Lond. Ser. B 276, 177–185.
Lutz, R.A., Jablonski, D., Turner, R.D., 1984. Larval development and dispersal at deep-sea hydrothermal vents. Science 226, 1451–1454.
Macaranas, J.M., Ablan, C.A., Pante, M.J.R., Benzie, J.A.H., Williams, S.T., 1992. Genetic structure of giant clam (*Tridacna derasa*) populations from reefs in the Indo-Pacific. Mar. Biol. 113, 231–238.
Maddison, W.P., Maddison, D.R., 2009. Mesquite: A Modular System for Evolutionary Analysis. Version 2.7.
Magsino, R., Juinio-Meñez, M., 2008. The influence of contrasting life history traits and oceanic processes on genetic structuring of rabbitfish populations *Siganus argenteus* and *Siganus fuscescens* along the eastern Philippine coasts. Mar. Biol. 154, 519–532.
McClain, C.R., 2007. Seamounts: identity crisis or split personality? J. Biogeography 34, 2001–2008.
McMillan, W.O., Palumbi, S.R., 1995. Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. Proc. R. Soc. Lond. Ser. B 260, 229–236.
Meier, R., Willman, R., 2000. The Hennigian species concept. In: Wheeler, W., Meier, R. (Eds.), Species Concepts and Phylogenetic Theory. University Press, New York, pp. 30–43.
Meyer, C.P., Geller, J.B., Paulay, G., 2005. Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. Evolution 59, 113–125.
Miller, K.G., Kominz, M.A., Browning, J.V., Wright, J.D., Mountain, G.S., Katz, M.E., Sugarman, P.J., Cramer, B.S., Christie-Blick, N., Pekar, S.F., 2005. The Phanerozoic record of global sea-level change. Science 310, 1293–1298.
Miyazaki, J.-I., Shintaku, M., Kyuno, A., Fujiwara, Y., Hashimoto, J., Iwasaki, H., 2004. Phylogenetic relationships of deep-sea mussels of the genus *Bathymodiolus* (Bivalvia: Mytilidae). Mar. Biol. 144, 527–535. doi:10.1007/s00227-003-1208.
Monaghan, M., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D.J.G., Lees, D.C., Ranaivosolo, R., Eggleton, P., Barraclough, T.G., Vogler, A., 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. Syst. Biol. 58, 298–311.
Moritz, C., 1994. Defining "evolutionary significant units" for conservation. Trends Ecol. Evol. 9, 373–375.
Newman, W.A., 1985. The abyssal hydrothermal vent invertebrate fauna: a glimpse of antiquity? Bull. Biol. Soc. Wash. 6, 231–242.
Nichols, R., 2001. Gene trees and species trees are not the same. Trends Ecol. Evol. 16, 358–364.
Nielsen, R., Wakeley, J., Nielsen, R., Slatkin, M., 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158, 885–896.
Nylander, J.A.A., 2004. MrModeltest v2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University, Uppsala.
O'Hara, T.D., 2007. Seamounts: centres of endemism or species richness for ophiuroids? Global Ecol. Biogeography 16, 720–732.
Olu-Le Roy, K., Cosel, R.v., Hourdez, S., Carney, S.L., Jollivet, D., 2007. Amphi-Atlantic cold-seep *Bathymodiolus* species complexes across the equatorial belt. Deep-Sea Res. Part I 54, 1890–1911.
Palumbi, S.R., 1997. Molecular biogeography of the Pacific. Coral Reefs 16, S47–S52.
Paulay, G., 1997. Diversity and distribution of reef organisms. In: Chapman, T., Hall, J. (Eds.), Life and Death of Coral Reefs. International Thomson Publishing, New York.
Pfenninger, M., Cordellier, M., Streit, B., 2006. Comparing the efficacy of morphologic and DNA-based taxonomy in the freshwater gastropod genus *Radix* (Basommatophora, Pulmonata). BMC Evol. Biol. 6, 100.
Planes, S., Fauvelot, C., 2002. Isolation by distance and vicariance drive genetic stucture of a coral reef fish in the Pacific ocean. Evolution 56, 378–399.
Plouviez, S., Shank, T.M., Faure, B., Daguin-Thiebaut, C., Viard, F., Lallier, F.H., Jollivet, D., 2009. Comparative phylogeography among hydrothermal vent species along the East Pacific Rise reveals vicariant processes and population expansion in the South. Mol. Ecol. 18, 3903–3917.
Pons, J., Barraclough, T., Gomez-Zurita, J., Cardoso, A., Duran, D., Hazell, S., Kamoun, S., Sumlin, W., Vogler, A., 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55, 595–609.
Qu, L.H., 1986. Structuration et évolution de l'A.R.N ribosomique 28S chez les Eucaryotes: étude systématique de la région 5' terminale. Paul Sabatier, Toulouse. p. 230.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. <http://tree.bio.ed.ac.uk/software/tracer/>.
Samadi, S., Barberousse, A., 2009. Species: towards new, well-grounded practices. Biol. J. Linn. Soc. 97, 217–222.
Samadi, S., Bottan, L., Macpherson, E., Richer de Forges, B., Boisselier, M.-C., 2006. Seamount endemism questioned by the geographic distribution and population genetic structure of marine invertebrates. Mar. Biol. 149, 1463–1475.
Samadi, S., Quemere, E., Lorion, J., Tillier, A., Cosel, R., Lopez, P., Cruaud, C., Couloux, A., Boisselier-Dubayle, M.-C., 2007. Molecular phylogeny in mytilids supports the wooden steps to deep-sea vents hypothesis. C. R. Biol. 330, 446–456.
Sibuet, M., Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Res. Part II 45, 517–567.
Sites, J.W., Marshall, J.C., 2004. Operational criteria for delimiting species. Ann. Rev. Ecol. Evol. Syst. 35, 199–227.

Smith, C.R., Baco, A.R., 2003. Ecology of whale falls at the deep-sea floor. In: Gibson, R.N., Atkinson, R.J.A. (Eds.), Oceanography and Marine Biology: An Annual Review, vol. 41. Taylor & Francis, London, pp. 311–354.
Smith, P.J., McVeagh, S.M., Won, Y., Vrijenhoek, R.C., 2004. Genetic heterogeneity among New Zealand species of hydrothermal vent mussels (Mytilidae: *Bathymodiolus*). Mar. Biol. 144, 537–545.
Southward, E.C., 2008. The morphology of bacterial symbioses in the gills of mussels of the genera *Adipicola* and *Idas* (Bivalvia: Mytilidae). J. Shellfish Res. 27, 139–146.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum Likelihood phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web-servers. Syst. Biol. 75, 758–771.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.
Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S., Fisher, M.C., 2000. Phylogenetic species recognition and species concepts in Fungi. Fungal Genet. Biol. 31, 21–32.
Van Dover, C.L., 2000. The Ecology of Deep-sea Hydrothermal Vents. Princeton University Press, Princeton.
Vogler, A.P., Monaghan, M.T., 2007. Recent advances in DNA taxonomy. J. Zool. Syst. Evol. Res. 45, 1–10.
Vrijenhoek, R.C., 2009. Cryptic species, phenotypic plasticity, and complex life histories: assessing deep-sea faunal diversity with molecular markers. Deep-Sea Res. Part II 56, 1713–1723.
Warén, A., 1993. New and little known Mollusca from Iceland and Scandinavia. Part 2. Sarsia 78, 159–201.
Warén, A., Carrozza, F., 1990. *Idas ghisottii* sp.n., a new mytilid bivalve associated with sunken wood in the mediterranean. Boll. Malacologico 26, 19–24.
Wheeler, Q., Meier, R., 2000. Species Concepts and Phylogenetic Theory: A Debate. Columbia University Press, New York.
Will, K.W., Mishler, B.D., Wheeler, Q.D., 2005. The perils of DNA barcoding and the need for integrative taxonomy. Syst. Biol. 54, 844–851.
Will, K.W., Rubinoff, D., 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics 20, 47–55.
Williams, S.T., 2007. Origins and diversification of Indo-West Pacific marine fauna: evolutionary history and biogeography of turban shells (Gastropoda, Turbinidae). Biol. J. Linn. Soc. 92, 573–592.
Williams, S.T., Duda, T.F., MacMillan, W.O., 2008. Did tectonic activity stimulate Oligo-Miocene speciation in the Indo-West Pacific? Evolution 62, 1618–1634.
Won, Y.-J., Jones, W.J., Vrijenhoek, R.C., 2008. Absence of cospeciation between deep-sea mytilids and their thiotrophic endosymbionts. J. Shellfish Res. 27, 129–138.
Won, Y., Hallam, S.J., O'Mullan, G.D., Vrijenhoek, R.C., 2003a. Cytonuclear disequilibrium in a hybrid zone involving deep-sea hydrothermal vent mussels of the genus *Bathymodiolus*. Mol. Ecol. 12, 3185–3190.
Won, Y., Young, C.R., Lutz, R.A., Vrijenhoek, R.C., 2003b. Dispersal barriers and isolation among deep-sea mussel populations (Mytilidae: *Bathymodiolus*) from eastern Pacific hydrothermal vents. Mol. Ecol. 12, 169–184.
Wullschleger, E.B., Jokela, J., 2002. Morphological plasticity and divergence in life-history traits between two closely related freshwater snails, *Lymnae ovata* and *Lymnae peregra*. J. Molluscan Stud. 68, 1–5.
Yeap, K.L., Black, R., Johnson, M.S., 2001. The complexity of phenotypic plasticity in the intertidal snail *Nodilittorina australis*. Biol. J. Linn. Soc. 72, 63–76.