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ORIGINAL ARTICLE

Out of sight, out of mind: high cryptic diversity obscures the identities and histories of geminate species in the marine bivalve subgenus *Acar*

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ABSTRACT

Aim The rise of the Isthmus of Panama and the formation of 'geminate' species pairs serves as an important model of allopatric speciation. However, to function as a model system, hypothesized geminates must first be shown to be each other's closest living relatives. If the presence of cryptic taxa obscures true relationships, the biogeographical histories of transisthmian taxa are likely to be misinterpreted. We have therefore completed a phylogeographic survey of the transisthmian bivalve subgenus *Acar* in the genus *Barbatia* to characterize patterns of tropical American diversity and to place transisthmian taxa in a regional phylogeographic context.

Location Tropical America.

Methods Mitochondrial cytochrome *c* oxidase I (COI) and nuclear internal transcribed spacer (ITS) sequences were obtained from 233 specimens of *Acar*. Sequences were analysed using cladistic and Bayesian methods. Divergence times between species were inferred from net nucleotide divergences and a coalescence-based method.

Results The survey revealed 22 COI clades that were also monophyletic at ITS, indicating that the taxonomy of *Acar* is potentially greater than a fivefold diversity underestimate. The lone previously recognized geminate [*Barbatia* (*Acar*) *gradata* and *Barbatia* (*Acar*) *domingensis*] is composed of 15 clades. Among the four transisthmian lineages identified, two diverged more than 14 Ma; the two other geminates split just prior to the time of final seaway closure. In addition to a fourfold increase in the number of known geminates, our data show that within-basin diversification has been more impressive, with one geminate splitting into five monophyletic clades in the Western Atlantic alone since seaway closure. Electron microscopy of the larval shells of specimens indicates that the transisthmian lineage with the greatest rate of post-Isthmian diversification possesses non-planktonic larvae, a life-history feature linked to high speciation rates.

Main conclusions Our analyses revealed that the identities of geminate pairs split by the Isthmus of Panama were obscured by extremely high tropical American cryptic diversity. Although we have identified four geminates, only two appear to have been split by the Isthmus. Our uncovering of extensive post-Isthmian diversification is consistent with the palaeontological perspective that the final closure of the Central American Seaway was followed by high rates of subgeneric diversification, particularly in the tropical Western Atlantic.

Keywords

Central American Seaway, coalescence, Isthmus of Panama, molecular clock, phylogeography, pseudo-congruence, sibling species, speciation, species diversity, vicariance.

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INTRODUCTION

The rise of the Isthmus of Panama and the closure of the Central American Seaway (CAS) is the most significant geological event to have shaped patterns of species diversity in tropical America over the last 20 Myr (Jackson & Budd, 1996; Allmon, 2001; and references within). On land, the formation of the Isthmus allowed intermingling of the terrestrial biotas of North and South America, but in the sea, the Isthmus severed all connections between the marine environments of the tropical Western Atlantic (WA) and Eastern Pacific (EP) oceans. Changes in ocean circulation associated with CAS closure in tropical America radically altered patterns of ocean circulation, seasonality and primary productivity (Glynn, 1982; Keigwin, 1982; Marra *et al.*, 1987; D'Croz *et al.*, 1991; Terranes *et al.*, 1996; Ibaraki, 1997; Haug & Tiedemann, 1998; Bartoli *et al.*, 2005; Groeneveld, 2005; Schneider & Schmittner, 2006), which in turn had massive impacts on species distributions, community composition, life-history evolution, and species diversity (e.g. Stanley, 1986; Vermeij & Petuch, 1986; Lessios, 1990; Allmon *et al.*, 1993; Jackson *et al.*, 1993; Jackson & Herrera Cubilla, 1999; Marko & Jackson, 2001; Marko & Moran, 2002; Todd *et al.*, 2002; Moran, 2004a; McAlister, 2008).

Sea-surface temperature and salinity data indicate that an episodic shallow-water marine connection between the tropical EP and WA persisted until *c.* 2 Ma (Cronin & Dowsett, 1996; Haug & Tiedemann, 1998; Bartoli *et al.*, 2005; Groeneveld, 2005), but from a biological perspective, the distinction in near-shore faunas between the two sides of the Isthmus is clearly evident by *c.* 3.5 Ma (Keller *et al.*, 1989; Duque-Caro, 1990a,b; Coates *et al.*, 1992; Collins *et al.*, 1996; Kameo & Sato, 2000). Because the geological closure of the CAS is a relatively well-dated and large-scale vicariant event, the physical separation of the tropical WA and EP oceans and the formation of so-called 'geminate' species (Jordan, 1908), sister-species found on either side of the Isthmus, provide a fundamentally important and widely cited model of allopatric speciation (Darwin, 1859; Vermeij, 1978; Coyne & Orr, 2004; Lessios, 2008). The true history of many geminates, however, is potentially more complex, given that many pairs show ancient molecular-based divergence times (e.g. Knowlton & Weigt, 1998). Although some variable molecular divergences can be attributed to a staggered history of geographical isolation among taxa with different ecological attributes and habitat preferences (Knowlton *et al.*, 1993; Knowlton & Weigt, 1998), when molecular clock studies and fossil histories are directly compared, some geminate pairs clearly have more convoluted biogeographical histories than can be explained by this relatively simple staggered model of allopatry (Marko & Jackson, 2001; Marko, 2002).

Three main factors can be invoked to explain this complexity. First, extensive post-Isthmian faunal turnover throughout tropical America following CAS closure 2–4 Ma probably obscures some sister-group relationships. Species turnover was particularly rapid in the Caribbean, where loss of subgeneric taxa was as high as 75% for some taxa (Stanley, 1986; Vermeij & Petuch, 1986; Allmon *et al.*, 1996; Jackson *et al.*, 1996). This remarkably high rate of extinction suggests that many nominal geminate pairs may not be true 'twins' formed by the rise of the Isthmus, but, instead, more distantly related taxonomic relicts persisting in the wake of a regional mass extinction that eradicated one or more members of true geminate pairs (Marko & Jackson, 2001).

A second factor is that comparisons of divergence times across co-distributed taxa may be biased by the near-exclusive use of sequences from single mtDNA genes to infer population or species splitting times of geminates (e.g. Bermingham & Lessios, 1993; Bermingham *et al.*, 1997; Knowlton & Weigt, 1998; Lessios, 1998; Lessios *et al.*, 2001; Marko, 2002; Marko & Moran, 2002). Bias arises here because the splitting times for gene copies, whose descendants have now come to characterize sister-species, will always pre-date speciation; if the geminate ancestral population size was large, speciation dates can be greatly overestimated as a result of the potentially deep coalescence of ancestral polymorphisms (Edwards & Beerli, 2000). Even if the ancestral population size was small, assessing the extent of inter-locus variation in estimates of gene splitting times is simply not possible with a single genetic marker (Hickerson *et al.*, 2006). Therefore, data from unlinked nuclear loci are essential for more accurate estimates of divergence times (Hare *et al.*, 2002; Jennings & Edwards, 2005).

The third and largely unresolved issue that may hamper reconstruction of the biogeographical histories of tropical American taxa is the spectre of sibling species, reproductively isolated taxa that are difficult to distinguish based on morphology (Mayr & Ashlock, 1991). When multiple lineages are wrongly lumped together, even the most basic biogeographical signatures of vicariance and dispersal will be difficult to detect, and patterns of speciation are likely to be misconstrued (Knowlton, 1993). Despite the fact that a handful of surveys in the Caribbean reveal numerous deep genetic breaks between regions and even adjacent islands (e.g. Taylor & Hellberg, 2003; Lee & Ó Foighil, 2004; Mathews, 2006), geographical variation within putative geminate lineages has rarely been considered (but see Lee & Ó Foighil, 2005). Thus, reconstruction of the biogeographical histories of transisthmian geminate lineages requires geographical sampling that is sufficient to identify species boundaries and establish the degree of phylogenetic relatedness among species clusters within the tropical American and adjacent regions.

In this study, we present a world-wide phylogeographic survey of a bivalve mollusc clade that, prior to our study, contained only a single known geminate species pair. Our study involves four nominal species of the genus *Barbatia* in the family Arcidae. Although *Barbatia* is polyphyletic (Marko, 2002), the four taxa considered here [*B. domingensis* (Lamarck, 1819), *B. gradata* (Broderip & Sowerby, 1829), *B. bailyi* (Bartsch, 1931), *B. plicata* (Dillwyn, 1817)] comprise the morphologically well-defined subgenus *Acar*, a monophyletic clade according to both nuclear and mitochondrial DNA

(mtDNA) phylogenies (Marko, 2002). We have gathered a combination of mtDNA (cytochrome *c* oxidase I) and nuclear internal transcribed spacer (ITS) sequence data from throughout these species' ranges to assess patterns of mitochondrial and nuclear DNA (nDNA) diversity as well as to provide estimates of divergence times of geminate species pairs. Our primary objective was to characterize patterns of phylogeographic differentiation in tropical American *Acar*, using nDNA to test patterns uncovered with mtDNA. Because complementary nDNA data has rarely been combined with mtDNA from geminates (but see Williams *et al.*, 2001; Ziegler & Lessios, 2004), our secondary objective was to generate divergence dates for living sister-species separated by the Isthmus of Panama, using markers from both genomes.

MATERIALS AND METHODS

Sampling and DNA sequencing

Between September 1997 and November 2006, specimens of *Barbatia* (*Acar*) were collected at 42 localities around the world (Table 1). Although we previously referred to specimens from the Philippines as '*B. divaricata*' (Marko, 2002), because we now cannot distinguish *B. divaricata* from *B. plicata* morphologically, we follow Oliver (1992) by referring to all Indo-Pacific specimens as *B. plicata*. We did not obtain specimens of *B. pulchella* (Reeve, 1844), a morphologically distinct member of the subgenus found in the Mediterranean (Oliver & von Cosel, 1992). Tissues were preserved in ethanol and shell material was saved for future analyses.

Genomic DNA was extracted using previously published methods (Marko, 2002). A fragment of mitochondrial cytochrome *c* oxidase I (COI) was amplified and sequenced using the primers HCOI and LCOI (Folmer *et al.*, 1994). In cases for which no amplification product was generated with this first primer pair, either arkLCOI (5′-TGGATTAAGGTTTCATATTCG-3′) was paired with HCOI or arkCOIF4 (5′-GGTGGGATTTAYAGDGABAYVCC-3′) and arkCOIR4 (5′-TAAGTATTAAYGTGMCYATCSGTWA-3′) were used as a pair. We also amplified a portion of the ribosomal ITS region using primers developed for *Mytilus* (Heath *et al.*, 1996). In *Acar*, these primers amplify an *c.* 1000-bp fragment that includes the ITS-1, 5.8S and ITS-2 regions. We subsequently designed an *Acar*-specific primer (5′-CCCAATGCGCAAGCAATT-3′) in the 5.8S ribosomal DNA (rDNA) region that, when paired with the first Heath *et al.* (1996) primer, yielded an *c.* 600-bp fragment of ITS-1 and 5.8S rRNA. Both genes were also amplified from *Arca imbricata* and *Arca ventricosa*, the outgroup taxa for phylogenetic analyses. A previous molecular phylogeny of the family Arcidae (Marko, 2002) showed that *Arca* is the sister-group to *Acar*, a result consistent with morphological analyses (Oliver, 1992).

COI amplifications were carried out as previously described (Marko, 2002) with annealing temperatures of 40–45°C. ITS amplifications were conducted in 50-μL reactions with 10 mM Tris, 50 mM KCl, 1.5 mM $MgCl_2$, 0.6 mM dNTPs, and 0.5 U of *Taq* DNA polymerase. Thermal cycling parameters for ITS were as follows: a denaturing step of 94°C for 60 s followed by 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s; cycles concluded with a 10-min incubation at 72°C. A 1°C s^{-1} ramp speed from the annealing temperature to the extension temperature was used for both genes. Amplification products were electrophoresed and purified with a kit (Qiagen, Valencia, CA, USA); products were then sequenced in both directions (Applied Biosystems, Foster City, CA, USA). Multiple peaks (suggesting the presence of more than one allele in ITS amplifications) were not observed, but some ITS amplifications yielded very poor sequence data and were subsequently cloned prior to sequencing.

Phylogenetic analysis

COI sequences were aligned easily owing to an absence of insertions and deletions (indels). Because of the presence of multiple small indels, ITS was first aligned with CLUSTAL version 1.83.1 (Chenna *et al.*, 2003) and subsequently checked by eye. Within *Acar*, the ITS alignment was simple, with the exception of three ingroup sequences (forming a monophyletic clade) and the outgroups, which were all highly divergent from the ingroup. We repeated the phylogenetic analyses with these divergent sequences excluded, but additional analyses did not yield trees that differed much from those built using all of the data. Inclusion of ITS gaps as characters also had little impact on the analyses, and we have therefore presented results only from analyses that ignored gaps. Using MODELTEST version 3.7 (Posada & Crandall, 1998) we determined that the General Time Reversible model with rate heterogeneity among sites and a fixed proportion of invariant sites (i.e. GTR + G + I) was the best-fitting substitution model for both loci.

To infer gene trees, we first constructed cladograms using a heuristic search under the optimality criterion of parsimony in PAUP* version 4.b10 (Swofford, 2002), with randomized addition (10 replicates) and tree bisection–reconnection (TBR) branch swapping. Robustness of clades was estimated by 1000 bootstrap replicates (Felsenstein, 1985) but with a 60-s time limit placed on each replicate. Bayesian phylogenetic inference using MRBAYES version 3.1 (Ronquist & Huelsenbeck, 2003) employed gene-specific models. Several initial runs were used to optimize search parameters, resulting in a final Markov chain Monte Carlo (MCMC) search with four chains of 2,000,000 steps each, sampling of trees every 100 steps, but with the first 4000 trees discarded.

Divergence times and gene flow

To estimate divergence times, we used two methods. First, with the best-fitting nucleotide substitution models and resulting patristic genetic distances, we calculated net nucleotide divergences between clades (Nei & Li, 1979), a method that takes into account ancestral polymorphism but assumes equal population sizes and no gene flow since the time of the split. We also inferred divergence times with the coalescent-based

Table 1 Summary of collection data for samples of *Barbatia* (*Acar*) *domingensis*, *B.* (*A.*) *gradata*, *B.* (*A.*) *bailyi* and *B.* (*A.*) *plicata* used for analyses of cytochrome *c* oxidase I (COI) and internal transcribed spacer (ITS) sequences.

Collection site*	Ocean basin†	Code‡	Nominal species	COI	ITS
Isla Taboga, Panama, Panama	EP	PAN	*B.* (*A.*) *gradata*	7	1
Isla Pericos, Panama, Panama	EP	PAN	*B.* (*A.*) *gradata*	3	1
Isla Del Rey, Panama, Panama	EP	PAN	*B.* (*A.*) *gradata*	1	0
Bahia Honda Chiriqui, Panama	EP	PAN	*B.* (*A.*) *gradata*	10	3
Isla Parida, Chiriqui, Panama	EP	PAN	*B.* (*A.*) *gradata*	15	5
Islas Secas, Chiriqui, Panama	EP	PAN	*B.* (*A.*) *gradata*	16	8
Isla Silva Afuera, Chiriqui, Panama	EP	PAN	*B.* (*A.*) *gradata*	18	4
Bahia Charco Azul, Chiriqui, Panama	EP	PAN	*B.* (*A.*) *gradata*	11	2
Playa Anclote, Puerto Vallarta, Mexico	EP	MEX	*B.* (*A.*) *gradata*	2	1
Bahia Suenos, Baja California Sur, Mexico	EP	MEX	*B.* (*A.*) *gradata*	13	2
Punta Chile, Baja California Sur, Mexico	EP	MEX	*B.* (*A.*) *gradata*	3	1
El Palmar, Baja California Sur, Mexico	EP	MEX	*B.* (*A.*) *gradata*	2	1
Punta Marquez, Baja California Sur, Mexico	EP	MEX	*B.* (*A.*) *gradata*	2	1
Los Frailes, Baja California Sur, Mexico	EP	MEX	*B.* (*A.*) *gradata*	2	1
Buena Vista, Baja California Sur, Mexico	EP	MEX	*B.* (*A.*) *gradata*	1	1
Punta Pequena, Baja California Sur, Mexico	EP	MEX	*B.* (*A.*) *gradata*	1	0
Playa Norte, Mazatlan, Sinaloa, Mexico	EP	MEX	*B.* (*A.*) *gradata*	1	1
Playa HoJo, Manta, Manabi, Ecuador	EP	ECU	*B.* (*A.*) *gradata*	16	9
Isla Marchena, Galapagos Archipelago, Ecuador	EP	ECU	*B.* (*A.*) *gradata*	2	0
Bahia Hachal, Guanacaste, Costa Rica	EP	CR	*B.* (*A.*) *gradata*	1	0
Crystal Cove, Newport Beach, CA, USA	EP	CA	*B.* (*A.*) *bailyi*	2	2
Viento Frio, Colon, Panama	WA	PAN	*B.* (*A.*) *domingensis*	10	2
Isla Grande, Colon, Panama	WA	PAN	*B.* (*A.*) *domingensis*	2	0
Cayo Nancy, Bocas Del Toro, Panama	WA	PAN	*B.* (*A.*) *domingensis*	9	3
Hospital Point, Bocas Del Toro, Panama	WA	PAN	*B.* (*A.*) *domingensis*	2	1
San Salvador Island, Bahamas	WA	BAH	*B.* (*A.*) *domingensis*	8	2
Triangles, Key Largo, FL, USA	WA	FL	*B.* (*A.*) *domingensis*	3	1
Discovery Bay, Jamaica	WA	JAM	*B.* (*A.*) *domingensis*	16	8
St John, United States Virgin Islands	WA	USVI	*B.* (*A.*) *domingensis*	8	7
Pigeon Point, Tobago	WA	TOB	*B.* (*A.*) *domingensis*	7	4
Speyside, Tobago	WA	TOB	*B.* (*A.*) *domingensis*	12	7
Radio Island, NC, USA	WA	NC	*B.* (*A.*) *domingensis*	2	2
Akumal, Quintana Roo, Mexico	WA	MEX	*B.* (*A.*) *domingensis*	1	1
Cabo Frio, Santa Cruz, Brazil	WA	BRA	*B.* (*A.*) *domingensis*	6	4
Houtman Abrolhos, Western Australia, Australia	IN	AUS	*B.* (*A.*) *plicata*	2	1
Dampier, Western Australia, Australia	IN	AUS	*B.* (*A.*) *plicata*	1	0
La Perouse Bay, Maui, Hawaii, USA	WP	HI	*B.* (*A.*) *plicata*	2	2
Makena Landing, Maui, Hawaii, USA	WP	HI	*B.* (*A.*) *plicata*	4	4
Shek-O, Hong Kong, China	WP	HK	*B.* (*A.*) *plicata*	2	2
Cape d'Aguilar, Hong Kong, China	WP	HK	*B.* (*A.*) *plicata*	1	0
Sao Vicente, Cape Verde Islands	EA	CV	*B.* (*A.*) *plicata*	2	1
Olango Island, Cebu, Philippines	WP	PHI	*B.* (*A.*) *plicata*	4	2

*Some localities consist of several sites separated by a few kilometres (e.g. 'Islas Secas'); additional collection information may be obtained from the authors.

†EP, Eastern Pacific; WA, Western Atlantic; IN, Indian Ocean; WP, Western Pacific; EA, Eastern Atlantic.

‡As used in Figures.

program IMa (Hey & Nielsen, 2004), which implements an MCMC search strategy to jointly estimate divergence time (t), migration rates (m) and genetic diversities (Θ) for two populations that share a common ancestor. Under a finite-sites model of sequence evolution (Hasegawa *et al.*, 1985), IMa jointly estimates these population genetic parameters in a Bayesian sampling framework by calculating posterior probabilities for parameters across a set of likely gene trees (Nielsen & Wakeley, 2001). For the prior distribution of t, we assumed that divergence times of geminates were between 0 and 20 Ma, assuming that unconstrained estimates of t would fall some time prior to *c.* 3 Ma. Short preliminary runs with variable numbers of chains were conducted to find an effective sampling strategy, followed by long runs (> 10,000,000 steps) with burn-in times of *c.* 20% of the total number of steps in the search. Convergence was assessed by monitoring trend plots,

effective sample sizes for *t*, and the consistency of results across 10 runs started with random seeds. Because this analysis assumes no recombination within loci, we first tested the ITS geminate data for evidence of recombination using IMGC (Woerner *et al.*, 2007), a program that uses violations of the '4-gamete test' (Hudson & Kaplan, 1985) to identify the largest recombination-filtered block of sequences.

Calculation of divergence times in years requires knowledge of mutation rates. Because one of our goals was to date the divergence of lineages separated by the Isthmus, we used 'local' clock rates derived from fossil-calibrated molecular data for the particular taxa we are studying (Marko, 2002). For COI, we used a divergence rate of 0.98% Myr^{-1} obtained from a comparison of three independent calibration points in the arcid fossil record (Marko, 2002) and converted this divergence rate to a gene-specific mutation rate for use with results from IMA. This COI rate is qualitatively similar to rates inferred from the fossil record of other molluscs (see Marko, 2002; and references within). For ITS, we inferred a divergence rate from the average sequence divergence between the subgenus *Acar* and the outgroup taxon *Arca*. *Acar* is the younger clade in the fossil record (see Marko, 2002), first appearing in the Danian Stage, the oldest division of the Palaeogene, *c.* 65 Ma (Reinhart, 1943). This split served as our ITS calibration point, yielding a divergence rate of 0.32% Myr^{-1}, similar to rates (0.39–1.32% Myr^{-1}) published previously (Savard *et al.*, 1993; Schlotterer *et al.*, 1994; Jobst *et al.*, 1998). Our rates (converted to per gene per year mutation rates) were then used to estimate separately the time of divergence for taxa using net nucleotide divergences at each locus, and the geometric mean of the two rates was used to convert the coalescent parameter *t* (scaled by mutation) from IMA into an estimate of divergence in years. Because IMA models mutation separately for each locus, we were also able to calculate *t* using the rate from one locus at a time combined with the mutation rate scalar that relates the mutation rate of individual loci to the geometric mean across all loci (Hey, 2007); this second method of converting *t* into years thus provides a way to compare the consistency of rate estimates between loci. The fossil calibration point for ITS was not one of the three used to calibrate COI (Marko, 2002), so the ITS calibration is completely independent of the COI rate estimate.

Gene flow (*Nm*, where *N* is the effective population size and *m* is the migration rate), the number of migrants entering each population or species each generation, was calculated between geminates by multiplying estimates of Θ (i.e. 4 $N\mu$, where μ is the mutation rate) for each recipient population by the corresponding estimate of the coalescent migration parameter *m* (i.e. m/μ) and dividing by four. Given the termination of the marine connection between the Atlantic and the Pacific *c.* 2 Ma, no gene flow is expected if a split dates to 2 Ma, whereas some gene flow is possible if lineages split prior to 2 Ma. For parameter estimates where unimodal posterior probability distributions both rose from and dropped to zero, 90% highest posterior density (HPD) intervals (i.e. credibility intervals) for parameter estimates were calculated.

RESULTS

Mitochondrial COI diversity

503 base pairs of COI could be scored from all 233 specimens. Among these, there were 298 phylogenetically informative sites. Given that only four taxa are recognized world-wide in *Acar*, the phylogeny based on mitochondrial COI (Fig. 1) shows unexpectedly high clade diversity in all regions sampled. The cladistic and Bayesian analyses produced similar topologies; both bootstrap percentages and posterior probabilities, respectively, provide good support for many clades.

As a starting point, we numbered all terminal monophyletic COI clades or lineages (in the case of single sequences) on the cladistic tree that were at least 3.0% divergent from a sister-clade or sequence. Although somewhat arbitrary, 3.0% represents *c.* 3 Myr of divergence at arcid bivalve COI (Marko, 2002), which corresponds roughly to the time of final CAS closure (see Introduction). In total, there are 28 clades at least 3% divergent from other clades in the COI phylogeny, and in most cases the divergence was far greater than 3% (Fig. 1).

Most of these '3% clades' are supported by both bootstrap values > 85% (cladistic analysis) and posterior probabilities > 0.90 (Bayesian inference), but three receive support from only one method. First, the cladistic analysis generated two sister-clades (WA1 and WA2) that are not reciprocally monophyletic in the Bayesian tree (Fig. 2a,b). Second, although monophyly of the EP1 clade receives good bootstrap support in the cladistic tree (89%), the clade is not quite monophyletic in the Bayesian tree (Fig. 3a,b). Third, cladistic and Bayesian trees differ with respect to the position of one sequence (WA10) from the Caribbean coast of Mexico; in this case, the Bayesian tree (Fig. 4b) shows reciprocally monophyletic EP and WA clades, but in the cladistic tree (Fig. 4a) the position of the WA10 lineage as the sister-lineage to the EP clade renders the WA lineages paraphyletic. However, the placement of the WA10 lineage is not well supported in either analysis.

The only nominal species of *Acar* recognized in the WA, *Barbatia* (*Acar*) *domingensis*, is wildly polyphyletic according to COI. The WA has a total of eleven 3% clades; some of these are closely related to each other (e.g. WA1 and WA2) whereas others are more closely related to clades in other regions (Fig. 1). The EP is similarly diverse at COI, with a total of nine 3% clades or lineages. Sequences from specimens from the type locality of *B. bailyi* in southern California (Bartsch, 1931; Moran, 2004b) form a very distinct lineage in Fig. 1 (EP5). However, other EP samples, which represent the nominal species *B. gradata*, are polyphyletic. West Pacific samples from Hawaii (WP1, WP3) and China (WP2) form a monophyletic clade that is the sister-group to a west Africa clade (EA1). An additional WP clade (WP4) from the Philippines is only distantly related to a large clade of EP and WA lineages. Samples from the Indian Ocean (IN1) show no close affinity to any other clades in the phylogeny, and their position in the tree is analysis-dependent.

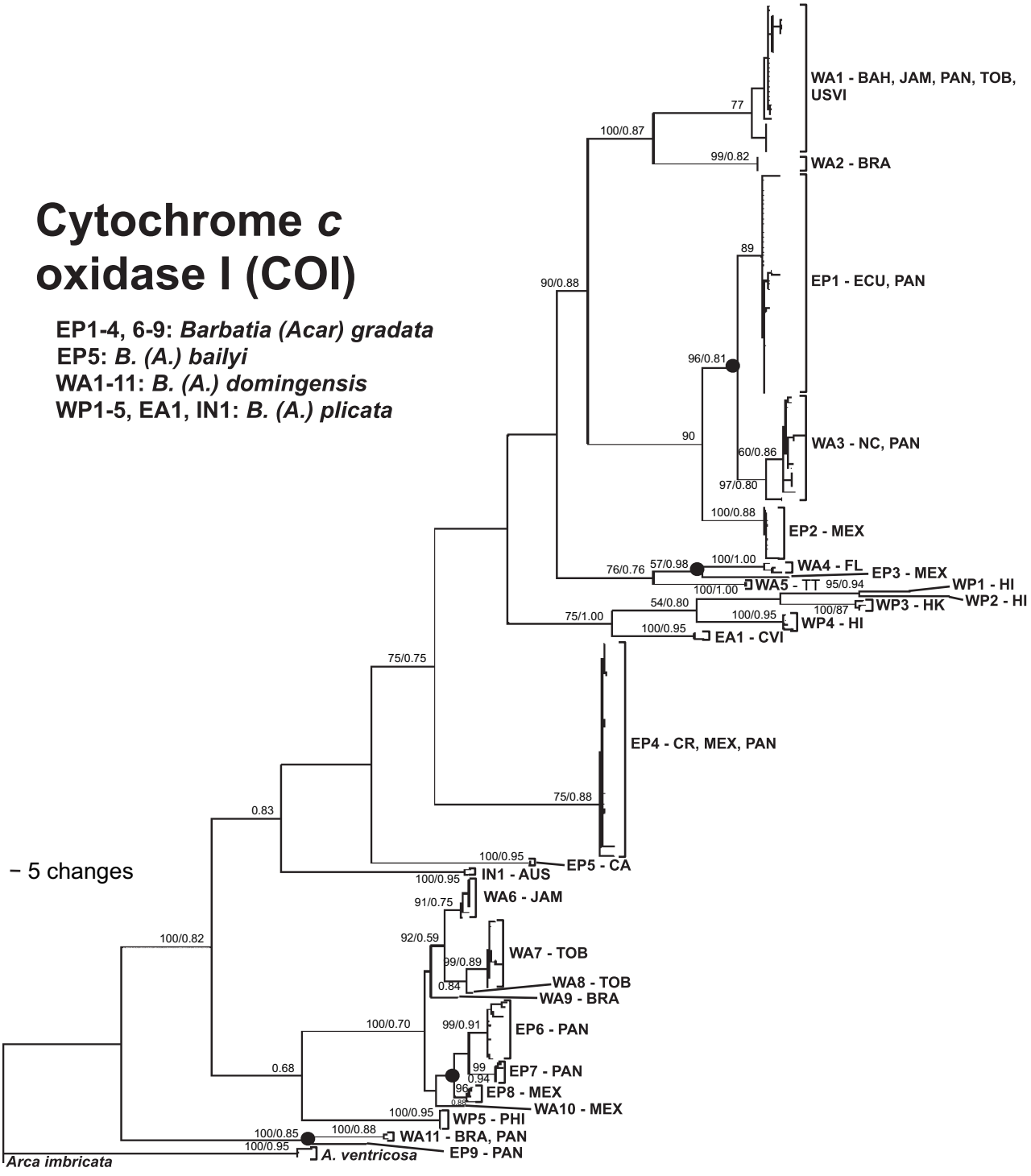

Figure 1 Phylogenetic tree for cytochrome *c* oxidase I (COI) haplotypes from samples of *Barbatia* (*Acar*) rooted with haplotypes from *Arca imbricata* and *A. ventricosa*. Numbers at nodes are bootstrap percentages and posterior probabilities from cladistic and Bayesian phylogenetic analyses, respectively. Clade names and regional codes are defined in Table 1. Closed circles at nodes denote geminate lineages. Sequences are deposited in GenBank under accession numbers FJ480451–FJ480683.

Although the current taxonomy recognizes only a single *Acar* geminate, our mtDNA survey revealed four sister-clades or lineages that are separated by the Isthmus of Panama: (1) EP1/WA3, (2) EP6-8/WA6-10, (3) EP9/WA11, and (4) EP3/WA4 (Fig. 1). The specimens thought to be geminates in previous studies (Marko & Jackson, 2001; Marko, 2002; Marko & Moran, 2002) are members of very distantly related clades (EP4 and WA1). Average COI sequence divergences (GTR + I + G corrected but not corrected for intraspecific diversities) between these 'new' geminate clades were 6.3, 10.3,

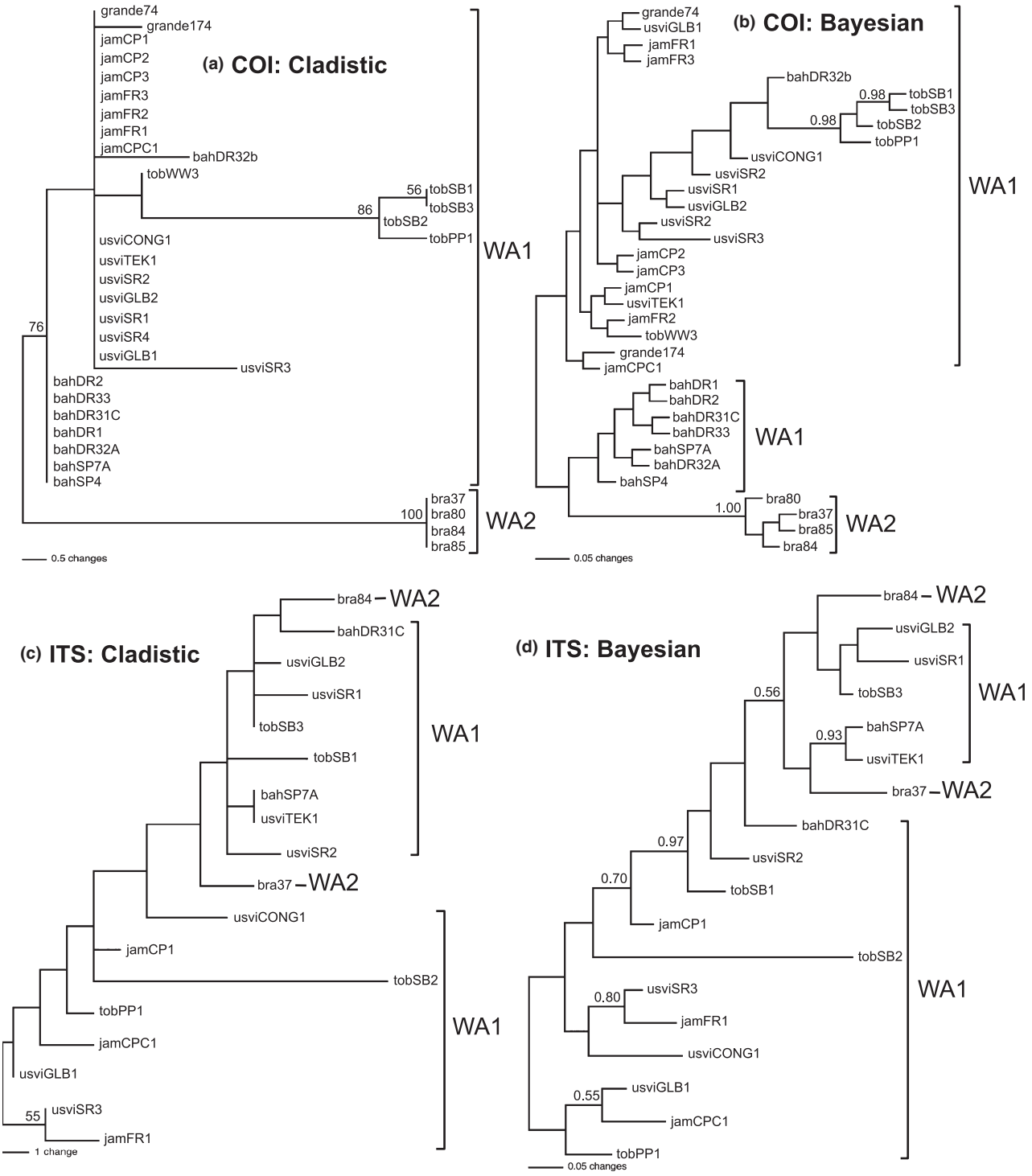

Figure 2 Phylogenetic trees for subclades of cytochrome *c* oxidase I (COI) haplotypes (a and b) and internal transcribed spacer (ITS) sequences (c and d) from samples of *Barbatia* (*Acar*). Labels as in Fig. 1. Trees were rooted with sister-lineages (not shown) in Figs 1 and 5.

19.1% and 26.5%, respectively. For the putative EP6-8/WA6-10 geminate pair (Fig. 4a,b), the apparent descendant EP and WA lineages each contained several 3% clades or lineages that are strongly supported by both bootstrap percentages and posterior probabilities.

Nuclear ITS diversity

Even though the ITS tree (Fig. 5) is very similar to that obtained from COI, it does not provide as much resolution with respect to terminal clades. For example, neither cladistic

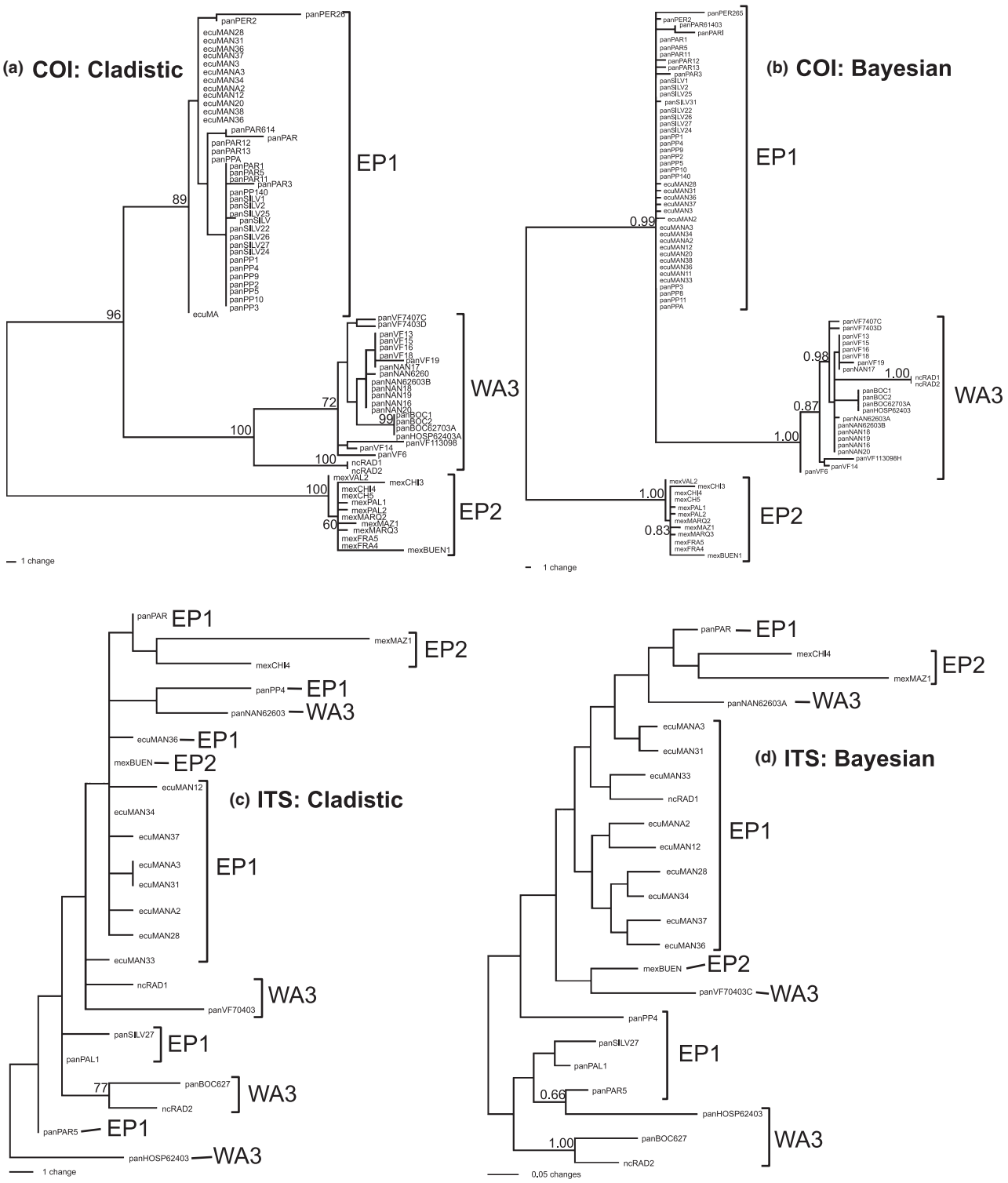

Figure 3 Phylogenetic trees for subclades of cytochrome *c* oxidase I (COI) haplotypes (a and b) and internal transcribed spacer (ITS) sequences (c and d) from samples of *Barbatia* (*Acar*). Labels as in Fig. 1. Trees were rooted with sister-lineages (not shown) in Figs 1 and 5.

nor Bayesian analyses recover distinct WA1 and WA2 clades (Fig. 2b,c); likewise, ITS sequences from the EP1/WA3 COI geminate clades are mixed together with EP2 sequences into a single large undifferentiated clade (Fig. 3c,d). Although the EP6-8/WA6-10 geminate pair is reciprocally monophyletic in the cladistic ITS tree (Fig. 4c,d), the EP6-8 clades are paraphyletic in the Bayesian ITS tree. The EP9/WA11 geminate pair is recovered as a sister-pair in the ITS tree (Fig. 5), but the

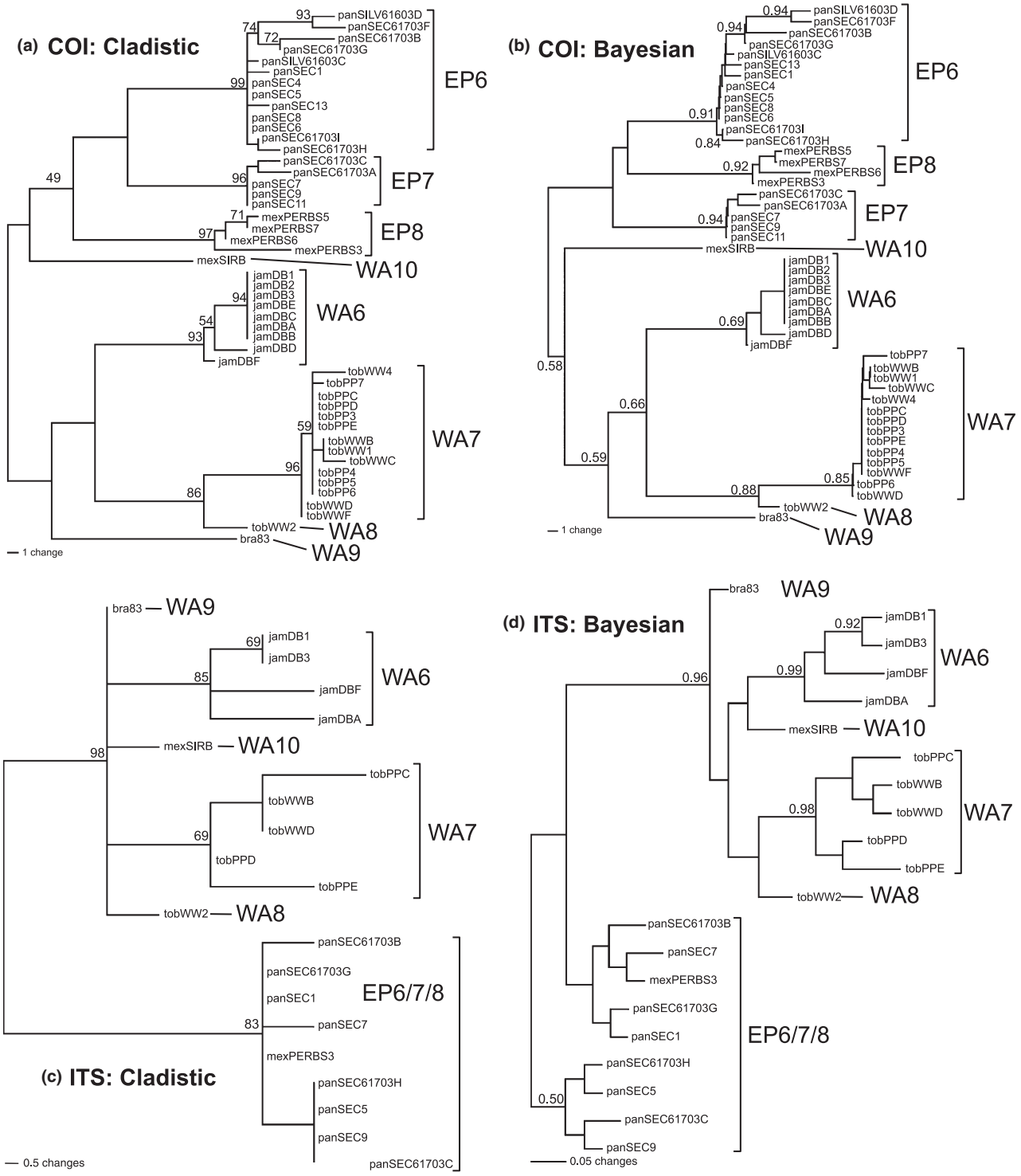

Figure 4 Phylogenetic trees for subclades of cytochrome *c* oxidase I (COI) haplotypes (a and b) and internal transcribed spacer (ITS) sequences (c and d) from samples of *Barbatia* (*Acar*). Labels as in Fig 1. Trees were rooted with sister-lineages (not shown) in Figs 1 and 5.

most divergent COI geminate pair (EP3/WA4) is not recovered as sister-taxa in the ITS tree; instead, the EP3 clade forms a geminate pair with both the WA4 and WA5 clades (Fig. 5).

Despite the differences, 22 of the 3% clades from the COI tree are present in the ITS tree; 15 of these clades are found in tropical America. Average transisthmian sequence divergences (GTR + I + G corrected, but not corrected with intraspecific diversities) for the geminate lineages recovered in the ITS tree are 1.9% (EP6-8/WA6-10), 3.5% (EP9/WA11) and 6.0% (EP3/WA4). Trees based on the combined COI and ITS data yield topologies (not shown) nearly identical to the ITS trees with respect to the terminal clades and recovery of geminate clades.

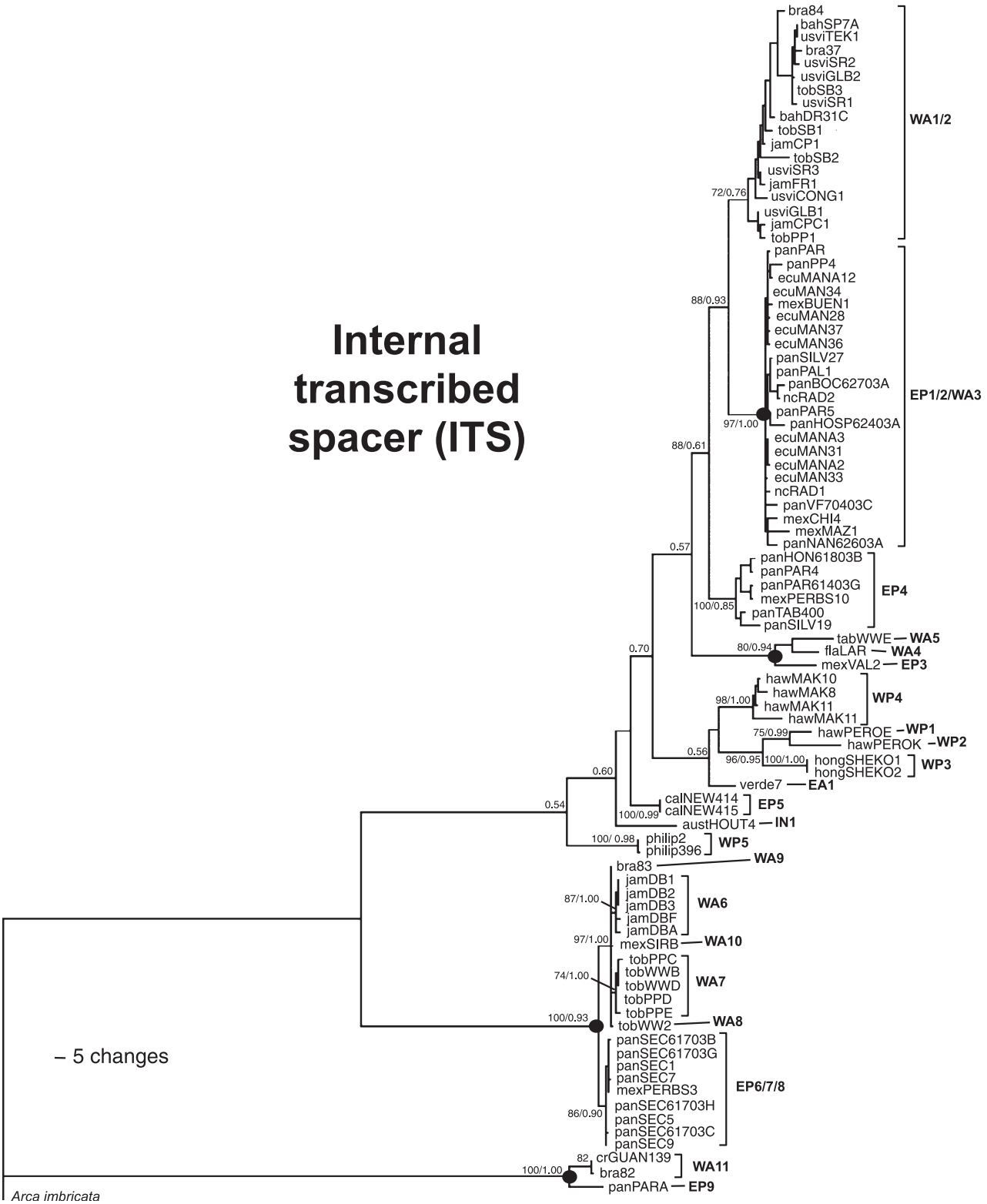

Figure 5 Phylogenetic tree for nuclear internal transcribed spacer (ITS) sequences from samples of *Barbatia* (*Acar*) rooted with haplotypes from *Arca imbricata*. Numbers at nodes are bootstrap percentages and posterior probabilities from cladistic and Bayesian phylogenetic analyses, respectively. Clade names and regional codes are defined in Table 1. Closed circles at nodes denote geminate lineages. Sequences are deposited in GenBank under accession numbers FJ480684–FJ480773.

Geographic distributions of tropical American clades

Some tropical American clades are widespread but many others are geographically restricted. For example, the WA1/WA2 clade is cosmopolitan in the WA and Caribbean, whereas the WA4 (Florida Keys) and WA5 (Tobago) are each found in only one place (Figs 1 and 5). The EP also contains a variety of geographically widespread (e.g. EP4) and restricted (e.g. EP2) clades (Figs 1 and 5). The two clades containing recently split transisthmian lineages show contrasting distributional patterns. First, nearly all nine clades within the phylogenetically diverse EP6-8/WA6-10 geminate lineage are restricted to single localities, but more than half (four of nine) show sympatric distributions with at least one other clade (Fig. 6). In contrast, members of the EP1-2 and WA3 clades are more broadly distributed, more abundant, but always allopatric within the WA and EP (Fig. 7). The most distantly related geminate clades (EP3/WA4 and EP9/WA11) are the rarest in our samples, with most found at only single sites (Fig. 8).

Divergence times between geminates

In the two relatively rare geminate pairs for which we obtained only small numbers of samples of each species (EP3/WA4 and EP9/WA11), we estimated divergence time only from net nucleotide divergences (NND, Table 2). According to COI NNDs, the EP9/WA11 pair split 17.4 Ma and the EP3/WA4 geminate pair split 27.0 Ma. Based on ITS, the same two geminates split 14.5 and 18.8 Ma, respectively.

For the two geminates for which samples within clades were large enough that divergence times could be inferred with IMa, estimates of t varied (Table 2). Neither of these two geminate

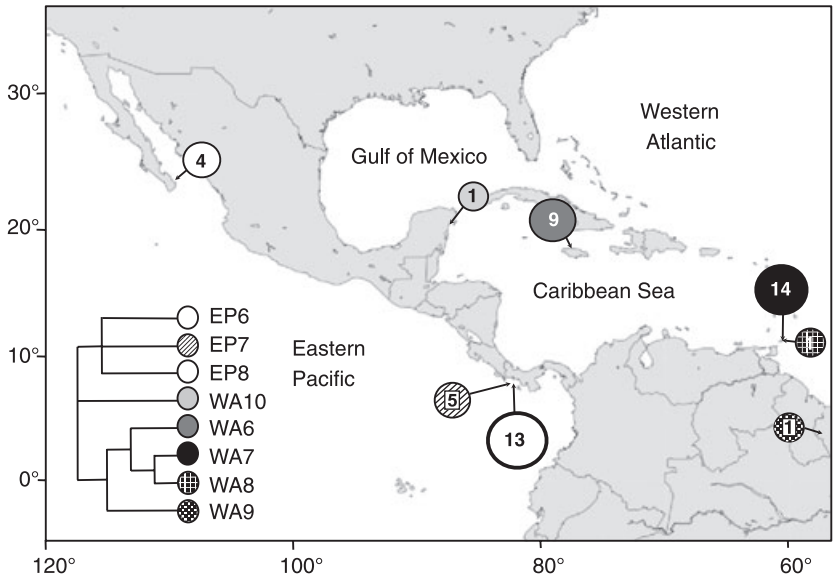

Figure 6 Spatial distribution of cytochrome *c* oxidase I (COI) haplotype clades for the EP6-8/WA6-10 transisthmian lineage of *Barbatia* (*Acar*). Numbers and the relative sizes of circles refer to sample sizes.

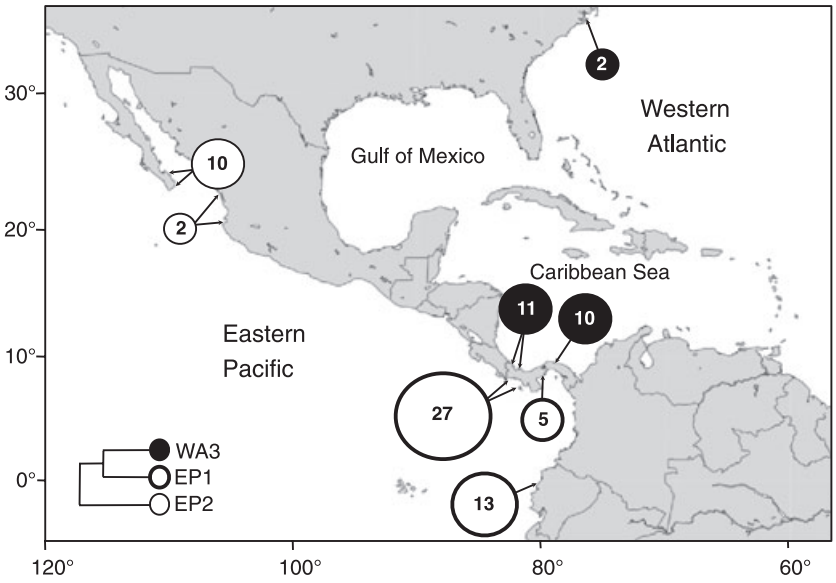

Figure 7 Spatial distribution of cytochrome *c* oxidase I (COI) haplotype clades for the EP1-2/WA3 transisthmian lineage of *Barbatia* (*Acar*). Numbers and the relative sizes of circles refer to sample sizes.

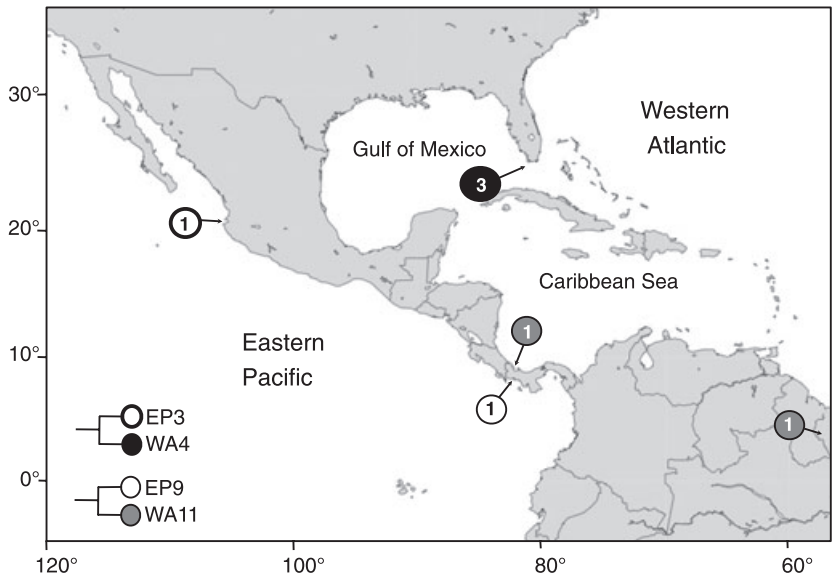

Figure 8 Spatial distributions of cytochrome *c* oxidase I (COI) haplotype clades for the EP3/WA4 and EP9/WA11 transisthmian lineages of *Barbatia* (*Acar*). Numbers and the relative sizes of circles refer to sample sizes.

ITS data sets showed violations of the 4-gamete test, so all of the ITS sequences and base pairs were included in this analysis. For the EP1/WA3 geminate, both the NND-based and IMa-based maximum likelihood estimates (MLEs) of *t* from COI (7.4 and 15.3 Ma, respectively) were substantially older than the time of final seaway closure. In contrast, the MLE of *t* from the combined COI/ITS data in IMa was far more recent (4.8 Ma). However, because the upper end of the posterior probability distribution for the combined COI/ITS estimate did not drop to zero (Fig. 9a), we could not calculate an upper bound on *t* (Table 2). Analysis of COI and ITS sequences from the EP6-8/WA6-10 geminate pair produced similar results, with the combined COI/ITS data set (Fig. 9b) yielding a MLE of *t* (3.3 Ma) that was much closer to the time of final seaway closure in comparison to estimates based on only COI (Table 2). More recent divergences from the combined mtDNA/nuclear data set were consistent with respect to the independently calibrated mutation rates of the loci, because we obtained very similar results (± *c.* 1 Myr, not shown) when we converted *t* into years using the mutation rate from only one locus at a time (see Methods).

For both geminates, the posterior probability distributions for *Nm* all show a sharp peak at the lowest limit of resolution in the frequency histogram generated by IMa (Fig. 9c,d), a result we interpret as meaning that the best estimate of *Nm* (in both directions) is zero for both geminates (see Won & Hey, 2005).

DISCUSSION

Our phylogeographic survey of the four nominal species that comprise the arcid bivalve subgenus *Acar* generated two main results. First, species diversity is undoubtedly underestimated for this subgenus. In tropical America, where only three nominal species of *Acar* are currently recognized, our survey detected 15 terminal clades that are monophyletic at both mitochondrial COI and nuclear ITS, suggesting the presence of numerous cryptic species. This lack of resolution with respect to species boundaries has completely obscured patterns of tropical American speciation, regional diversity, and endemicity. Second, rather than containing a single geminate species pair separated by the Isthmus of Panama, tropical American

Table 2 Estimates of geminate divergence times from *Barbatia* (*Acar*) based on net nucleotide divergence (NND) and the isolation-with-migration model (IMa). 90% highest posterior density (HPD) intervals for divergence times (years) and gene flow (*Nm*) are provided (when possible, see Materials and Methods) for maximum likelihood estimates (MLEs) yielded by IMa.

	CO1 divergence		CO1/ITS divergence	Gene flow	
Geminate pair	NND	IMa	IMa	To EP	To WA
(a) EP1/WA3					
MLE	7,400,000	15,345,000	4,821,000	0	0
Upper 90% HPD	–	–	–	–	–
Lower 90% HPD	–	–	726,000	–	–
(b) EP6-8/WA6-10					
MLE	5,400,00	7,931,000	3,280,000	0	0
Upper 90% HPD	–	–	–	–	–
Lower 90% HPD	–	3,001,000	2,038,000	–	–

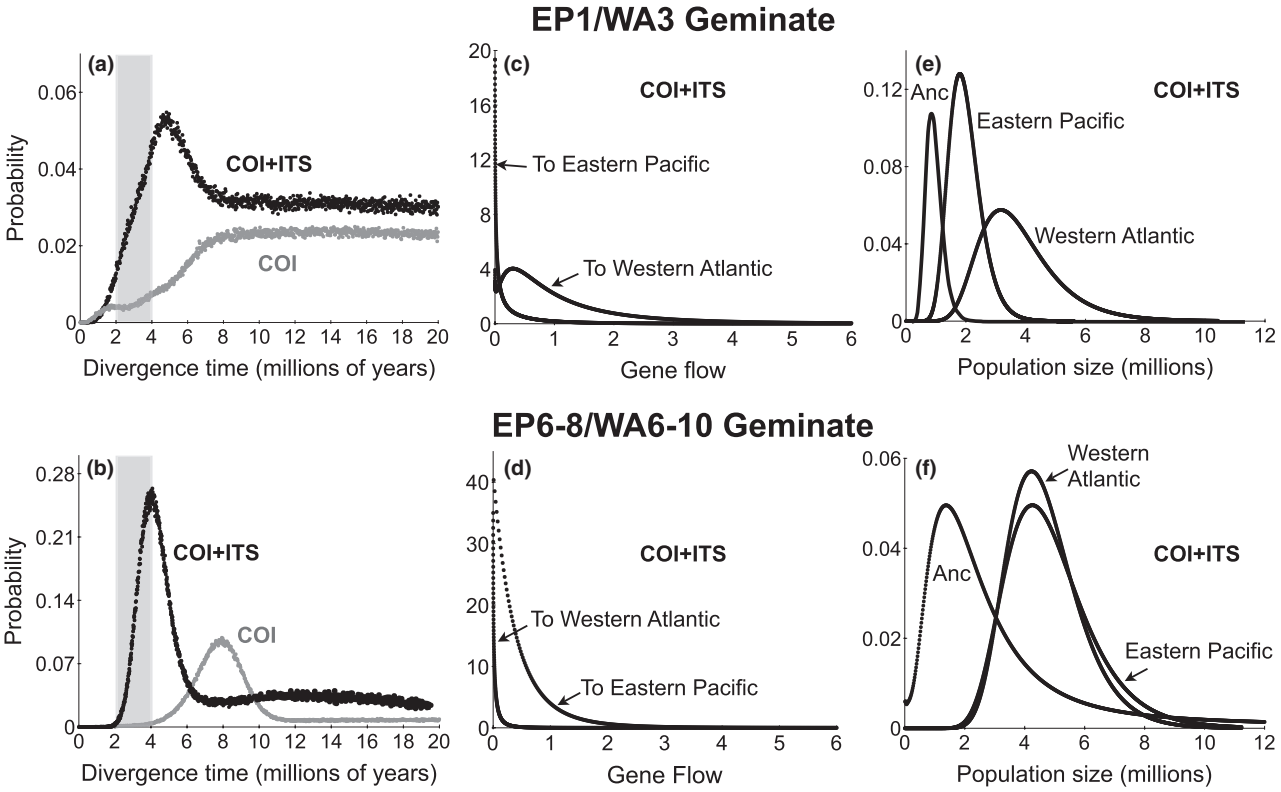

Figure 9 Posterior probability distributions for divergence time, gene flow and effective population size for the EP1/WA3 and EP6-8/WA6-10 transisthmian lineages of *Barbatia* (*Acar*). Divergence time distributions (panels a and b) are inferred from cytochrome *c* oxidase I (COI) and COI combined with internal transcribed spacer (ITS); estimates of other parameters (panels c–f) are based on the two genes combined. Anc, ancestral population size. The shaded box (in a and b) marks the last 2 Myr prior to final seaway closure (at 2 Ma).

Acar harbours at least four cryptic transisthmian species pairs, ranging in age from *c.* 3 to > 14 Ma.

Mitochondrial and nuclear clade diversity

Our world-wide mtDNA survey revealed the existence of 28 monophyletic clades that are at least 3% divergent from their closest relative in the COI phylogeny. Because the mtDNA genome is haploid and maternally inherited, stochastic lineage sorting (with no gene flow) resulting in reciprocal monophyly is expected to occur on the order of *c.* *N* generations, where *N* is the effective population size (Birky, 1991). Although *N* could be large for many out-crossing, broadcast spawning marine species with widely dispersing planktonic larvae, it would be premature to propose new species based only on mtDNA. However, 22 of these 28 3% divergent COI clades are also monophyletic at the unlinked nuclear ITS locus. Given that the 'time to monophyly' for a nuclear locus is roughly four times greater than that for mtDNA (Kimura & Ohta, 1969; Tajima, 1989), the congruent patterns of monophyly between mitochondrial and nuclear gene trees in *Acar* imply fairly ancient separations among these 22 clades.

Given the high stochasticity of the coalescent process, such a large number of coincident terminal mitochondrial and nuclear clades in *Acar* is highly unlikely just as a result of chance (Moore, 1995; Neigel & Avise, 1986; Hudson & Turelli, 2003). This suggests to us that this subgenus harbours at least 22 cryptic species, 15 of which are in tropical America. With respect to the biological species concept, this is probably a conservative estimate given that many recently diverged (reproductively isolated) species are not yet reciprocally monophyletic at molecular markers (Funk & Omland, 2003; Hart & Sunday, 2007). From this perspective, it is worthwhile to note that, despite *c.* 3 Myr of geographical isolation on either side of the Isthmus, one of our putative geminate pairs (EP1/WA3) is only reciprocally monophyletic at COI in some analyses (Fig. 3a vs. b) and is very far from reciprocal nuclear monophyly at nuclear ITS (Fig. 3c,d).

Neither high diversity nor high regional endemism of clades within *Acar* is completely unexpected. Previous morphological analyses on a subset of the shells from samples analysed here showed that morphometric distances between samples within each of the WA and EP are larger on average than those across the Isthmus (Marko & Jackson, 2001). In discriminant morphological analyses in which collection site was the classification group *a priori*, 90% of EP and 83% of WA specimens were correctly classified to their collection site (Marko & Jackson, 2001). We have repeated that analysis on the subset of specimens from Marko & Jackson (2001) with individuals grouped based on their membership in mtDNA/nDNA clades identified in the present study: this revised analysis showed correct assignments for 97% (EP) and 96% (WA) of specimens to their molecular

clades (P. B. Marko, unpublished data). Although a complete morphometric analysis of all of the shells used in the present study is in progress, this preliminary re-analysis indicates that many of the mtDNA/nDNA clades characterized here are probably also morphologically distinct.

On larger spatial scales, conclusions about phylogenetic affinities are tentative at best, given our somewhat limited sampling outside tropical America. However, what data we have suggest few recent biotic connections between any major biogeographical regions. Most notable is the lack of any recent connection across the tropical Atlantic, which has exchanged numerous molluscan lineages over the last 5 Myr (Vermeij & Rosenberg, 1993; Vermeij, 2004, 2005). Unlike species in the cool-temperate north-western Atlantic (see Wares & Cunningham, 2001), which suffered significant regional extinctions during Pleistocene glacials, the inhabitants of the tropical north-western Atlantic are clearly not recently derived from eastern Atlantic populations. Our samples from west Africa (EA1) are in fact most closely related to a large west Pacific clade, whose COI (23%, 24 Ma) and ITS (8%, 25 Ma) divergences both coincide with the closure of the Tethys seaway 26 Ma (Ricou, 1987, 1994). Therefore, the phylogeographic patterns observed in *Acar* are consistent with the tropical American region containing numerous anciently derived endemic lineages that have been diversifying *in situ* for millions of years, a pattern similar to that reported in WA scleractinian corals (Fukami *et al.*, 2004; Nunes *et al.*, 2008).

Divergence of geminate lineages

Previous molecular work on arcids uncovered only a single geminate pair in *Acar*, which diverged > 20 Ma according to COI sequences calibrated from the fossil record (Marko, 2002). Based on our results here, our previous work clearly suffered from being conducted using the same assumption that probably plagues other molecular studies of geminates: that single specimens collected on either side of the Isthmus are in fact sister-taxa split by the rising Isthmus 2–4 Ma.

Our geographical sampling of the tropical WA and EP reveals a total of four putative *Acar* geminate clades isolated by the Isthmus. Divergence times for all four are variable, providing an example of the biogeographical phenomenon of pseudo-congruence (Cunningham & Collins, 1994; Riddle, 2005; Lomolino *et al.*, 2006). Two of these pairs (EP9/WA11 and EP3/WA4 or EP3/WA4-5), which were relatively rare in our collections, appear to represent such ancient splits (> 14 Ma) that the Isthmus cannot be implicated in their divergence, given that the earliest disruption of deep-water exchange is not detected in the geological record until the middle Miocene (Duque-Caro, 1990a; b; Coates & Obando, 1996). For the other two geminate pairs, MLEs for population splitting times (t) are consistent with geographical isolation having occurred shortly before final seaway closure.

Molecular clock calibration for geminates is a contentious issue (Lessios, 2008), but the best way to assess calibrations is through comparison involving different loci and different calibration points. It is therefore important to note that, whether our IMa analyses were calibrated with COI or ITS, IMa yields similar divergence time estimates for the two most recently split geminates. Any molecular clock, however, is likely to 'tick' too fast, given that the actual speciation event at a calibration node will always pre-date the first appearance of morphologically divergent calibration taxa in the fossil record (Marko, 2002); our estimates are thus probably best viewed as closer to lower bounds rather than upper bounds on estimates of t.

Compared with the combined analyses of mitochondrial and nuclear data, analysis of mitochondrial COI alone appears to yield more ancient estimates of t. Even though the upper bounds on the MLEs for t remain enormous with two loci, the MLEs for t from the combined COI/ITS data sets are less than half that of the estimates based only on COI. This result was consistent when we used either of the two genes to convert population splitting times scaled by mutation into values expressed in years, indicating that this result is not caused by an erroneously 'fast' ITS rate. In the most general sense, this result reflects the high stochastic variance of the coalescent, such that the addition of any additional locus will yield a different, and presumably more accurate, estimate of t (Edwards & Beerli, 2000; Jennings & Edwards, 2005). This result probably also reflects the fact that mtDNA genealogies, owing to the fourfold smaller effective population size (N) of mtDNA, retain less information about demographic parameters in the past, such as ancestral N, which are necessary for accurate estimates of population splitting times. Specifically, the smaller N for an mtDNA locus results in a more rapid loss of polymorphism and approach to reciprocal monophyly. Reciprocal monophyly, however, is consistent with either a relatively ancient population splitting time or a relatively large ancestral N; given that both recently diverged geminates are reciprocally monophyletic at COI (or nearly so), these single-locus data sets probably lack enough information to independently resolve both t and ancestral N.

An important caveat for consideration when dating divergence times of populations with the isolation-with-migration model is the potential impact of population subdivision. Because the expected coalescence for randomly drawn alleles increases with greater population subdivision (Takahata & Tajima, 1991; Nei & Takahata, 1993; Hoelzer, 1997), subdivision is expected to inflate estimates of t as well as the interlocus variance in t (Edwards & Beerli, 2000). Therefore, if transisthmian lineages have a history of subdivision, particularly in the ancestral population, our estimates of t may be biased upwards. This is potentially most problematic for the EP6-8/WA6-10 geminate lineage, which appears to be spatially structured, probably consisting of several cryptic species. However, given that the inferred divergence time for this clade (3.3 Ma) is so close to the time of final seaway closure (*c.* 2 Ma), any upward bias in t cannot be large. One possibility is that, even though some of the living geminate clades are currently spatially structured, the ancestral population for

these geminates may not have been as subdivided. Regardless, we conducted a supplementary IMa analysis in which we estimated pairwise divergence times within the EP6-8/WA6-10 lineage, in which we included only one clade from either side of the Isthmus at a time (e.g. EP6 and WA7) to reduce the population subdivision bias. However, all of the estimates from these pairwise comparisons are either similar to or slightly older than the divergence time estimate using all of the clades in the EP6-8/WA6-10 lineage (not shown), suggesting that the bias of population subdivision on divergence time estimates is minimal for our data.

Although the isolation-with-migration model estimates the rate of gene flow (*Nm*) following the separation of two populations, our results are most consistent with a scenario of allopatric speciation across the Isthmus in which no gene flow occurred following the split. For the EP1/WA3 geminate, the posterior probability distribution for *Nm* from the EP to the WA shows a second smaller peak at a value of $Nm \sim 0.4$ (Fig. 9c), hinting at the possibility of some gene flow after the initial separation of Atlantic and Pacific populations. Considering that a marine connection between the two oceans may have persisted up until *c.* 2 Ma (Keller *et al.*, 1989; Cronin & Dowsett, 1996; Haug & Tiedemann, 1998; Bartoli *et al.*, 2005; Groeneveld, 2005), genetic exchange occurring between the time of population separation (4.8 Ma) and the complete cessation of water flow between the WA and EP (2 Ma) is possible. However, this result may also simply reflect the inability of IMa to distinguish ancestral ITS polymorphisms from more recent gene flow, highlighting the need for more data from other unlinked loci.

Within-basin diversification

Although the gradual division of the tropical American marine environment by the rise of the Isthmus of Panama garners considerable attention as a textbook example of allopatric speciation (e.g. Freeman & Herron, 2007), it is increasingly apparent that the reality of the geminate species concept is made more complex by speciation events that occurred on smaller spatial and temporal scales than events directly associated with the closure of the CAS. Our phylogenies show

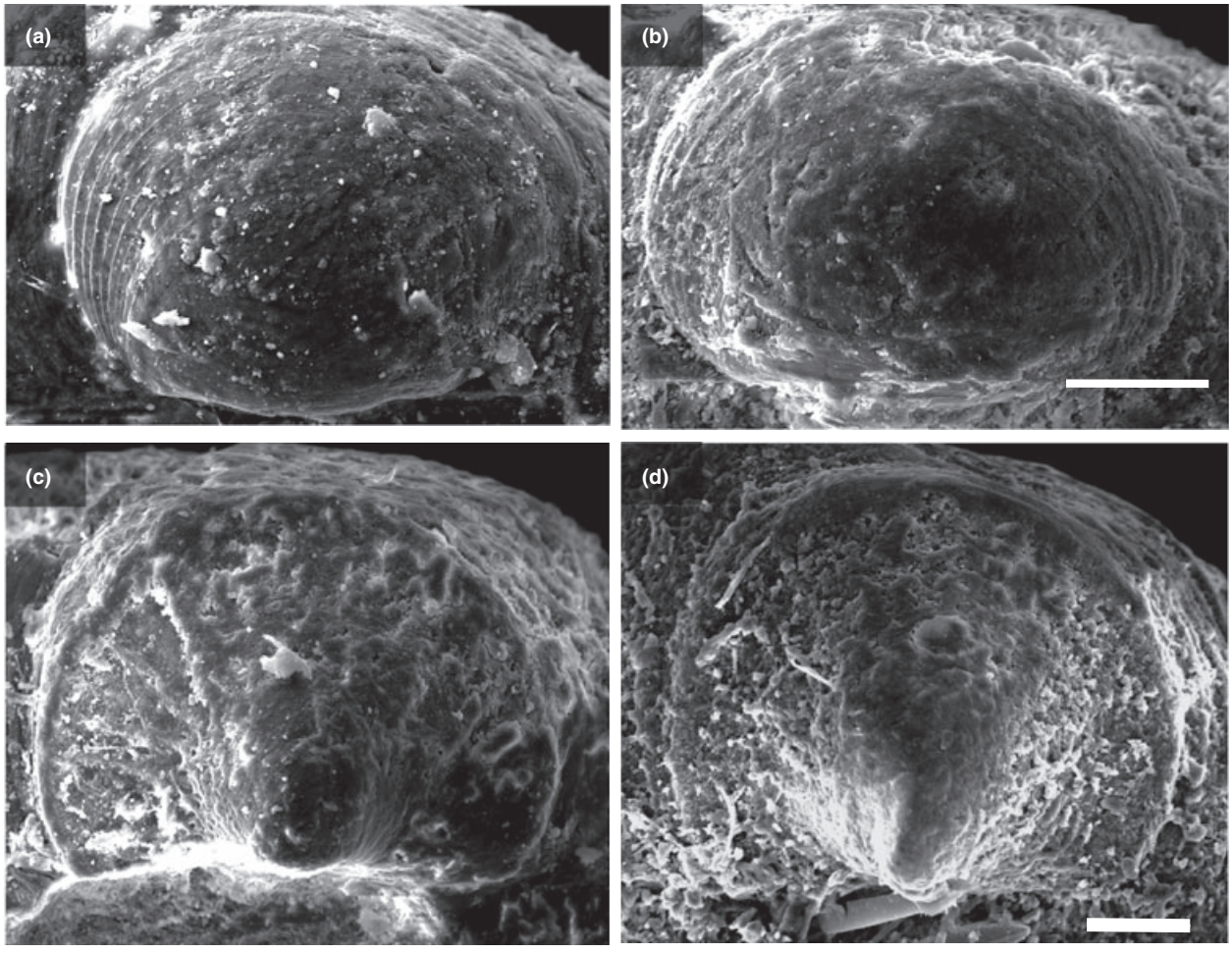

Figure 10 Scanning electron micrographs of the prodissoconchs of geminate lineages *Barbatia* (*Acar*) showing developmental modes. (a) Caribbean planktotroph (panVF7403D, lineage WA3); (b) Pacific planktotroph (ecuMAN31, lineage EP1); (c) Caribbean non-planktotroph (jamDBF, lineage WA6); (d) Pacific nonplanktotroph (panSEC7, lineage EP7). Scale bars, 50 μm (a = b, c = d).

coincident COI/ITS monophyly of five WA clades in the EP6-8/WA6-10 geminate 'pair' that all post-date the transisthmian split. If we assume that these monophyletic clades represent reproductively isolated species, then new species have arisen in the WA at a rate of > 1 Myr^{-1}. This pattern of rapid post-Isthmian cladogenesis is consistent with palaeontological evidence that the WA underwent a massive turnover event *c.* 2 Ma when primary productivity in the Caribbean dropped in response to complete CAS closure and re-organization of tropical WA ocean circulation (Allmon, 2001; and references within). The same high rate of diversification is not evident in the EP, although the COI data suggest that the EP6-8 clade has also undergone some post-Isthmian diversification (Fig. 4a,b).

The two geminates that appear to have split close to the time of final seaway closure show strongly contrasting patterns of both diversification and geographical distribution. The EP6-8/WA6-10 clade is diverse, yet individual clades are geographically restricted, with each clade restricted to a single locality. In contrast, the EP1/WA3 lineage contains only two members, both of which are much more broadly distributed within their respective oceans. What could cause such striking differences in diversification and distributional patterns between different geminate lineages? Larval life histories have been strongly implicated in both rates of diversification and geographical range extent: in general, species with planktonic, planktotrophic (feeding) larvae tend to have higher dispersal capabilities and thus larger geographical ranges and lower speciation rates than species with non-dispersive benthic, or brooded larvae (Thorson, 1950; Hansen, 1978, 1980; Jablonski & Lutz, 1983; Bhaud, 1993; Duda & Palumbi, 1999; Jeffery & Emlet, 2003). We therefore used scanning electron microscopy to examine the prodissoconchs (larval shells) from specimens from each of the two geminate lineages in question. The prodissoconch is the embryonic and larval bivalve shell formed during early development, and it is well established that morphological features of prodissoconchs can be used to infer both egg size and larval developmental mode (Ockelmann, 1965; Goodsell & Eversole, 1992; Moran, 2004b). Our micrographs show clearly that prodissoconchs of the less diverse and more broadly distributed geminate lineage (EP1/WA3) have large areas of ribbed accretionary growth consistent with planktotrophic development (Fig. 10a,b). In contrast, shell samples from the more diverse geminate lineage (EP6-8/WA6-10), whose members appear to have relatively narrow geographic distributions, have a larval shell characteristic of non-planktotrophic brooded development (Fig. 10c,d). Thus, the limited larval dispersal capability of the EP6-8/WA6-10 clade may be an important factor in limiting species range extents in this lineage, and in driving higher rates of diversification than are seen in planktotrophic lineages.

CONCLUSIONS

Although the characterization of patterns of species diversity and species distributions is essentially a descriptive exercise, the reconstruction of biogeographical histories relies fundamentally on this most basic of information (Knowlton, 1993). Our phylogeographic survey of what was thought to be a single transisthmian lineage clearly shows that casual collection of small numbers of specimens on either side of the Isthmus of Panama can easily lead to the misidentification of sister-taxa split by the Isthmus, and to misinterpretation of the evolutionary histories of these model biogeographical taxa. For example, in the case of *Acar*, we unwittingly assigned a 'geminate' divergence time of > 25 Ma for the *B. gradata/B. domingensis* species pair (Marko, 2002) using samples from lineages that we now know are not sister-taxa. Nevertheless, even when true transisthmian sister-taxa were identified, two of these sister-taxa were still relatively anciently diverged (> 14 Ma) relative to the time of final seaway closure. The combination of data from COI and ITS provides better resolution with respect to divergence times than COI alone, but it is still clear that several additional loci will be needed to obtain more precise estimates of these divergence times.

Geographic sampling across the entire tropical American region (and elsewhere) also revealed a complex and unprecedented pattern of concordant mitochondrial and nuclear diversification, particularly in the WA. Despite the fact that geminates are viewed as a model system for allopatric speciation, the apparent rapid molecular diversification of *Acar* on both sides of the Isthmus is consistent with the hypothesis that post-Isthmian faunal turnover within the EP and WA had a greater impact on biotic diversity than the divisive effects of the Isthmus itself (Jackson & Budd, 1996). Given that the relatively rapid post-Isthmian accumulation of lineages in *Acar* appears to be greatest in a clade whose members lack dispersive larvae, our results also suggest that post-Isthmian faunal turnover was selective with respect to larval developmental mode.

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BIOSKETCHES

Peter Marko's research uses molecular phylogeographical and palaeontological approaches to address questions related to biogeography, speciation, climate change and conservation.

Amy Moran's research focuses on patterns of species diversity in marine environments and life-history evolution, with an emphasis on physiological and morphological adaptations in early life stages to different environmental conditions.

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