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Genetic diversification and demographic history of the cactophilic pseudoscorpion *Dinocheirus arizonensis* from the Sonoran Desert

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ABSTRACT

Sequence data from a segment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene were used to examine phylogenetic relationships, estimate gene flow and infer demographic history of the cactophilic chernetid pseudoscorpion, *Dinocheirus arizonensis* (Banks), from the Sonoran Desert. Phylogenetic trees resolved two clades of *D. arizonensis*, one from mainland Sonora, Mexico and southern Arizona (clade I) and the other from the Baja California peninsula and southern Arizona (clade II). The two clades were separated by a mean genetic distance (*d*) of ~2.6%. Hierarchical analysis of molecular variance indicated highly significant population structuring in *D. arizonensis* (overall $\Phi_{ST} = 0.860$; $P < 0.0001$), with 80% of the genetic variation distributed among the two clades. Most pairwise comparisons of Φ_{ST} among populations within each clade, however, were not significant. The results suggest that phoretic dispersal on vagile cactophilic insects such as the neriid cactus fly *Odontoloxozus longicornis* (Coquillett) provides sufficient gene flow to offset the accumulation of unique haplotypes within each clade of the non-vagile pseudoscorpion. Preliminary results on dispersal capability of *O. longicornis* were consistent with this conclusion. Tests designed to reconstruct demographic history from sequence data indicated that both clades of *D. arizonensis*, as well as *O. longicornis*, have experienced historical population expansions. Potential barriers to gene flow that may have led to genetic isolation and diversification in clades I and II of *D. arizonensis* are discussed.

1. Introduction

Necrotic tissues (rots) of several species of columnar cacti from the Sonoran Desert of southwestern USA and northwestern Mexico, including saguaro (*Carnegiea gigantea*), cardón (*Pachycereus pringlei*), organ pipe (*Stenocereus thurberi*), agria (*S. gummosus*) and senita (*Lophocereus schottii*), provide an ideal environment for the feeding, breeding and development of a host of insects and other arthropods (Ryckman and Olsen, 1963; Castrezana and Markow, 2001). Although the availability of suitable microhabitat for the cactophilic arthropods varies with cactus species, in all cases necroses are distributed in a patchy manner and are ephemeral, with an individual rot lasting anywhere from a few weeks for senita to several months for saguaro and cardón (Breitmeyer and Markow, 1998). Thus, the ability to disperse to fresh necroses is of fundamental importance for the survival of organisms dependent upon this microhabitat. In addition, the different life histories and dispersal abilities of the diverse community of cactophilic arthropods provide a unique opportunity to examine how these traits might interact to influence the evolutionary histories of organisms that share the same habitat.

The chernetid pseudoscorpion, *Dinocheirus arizonensis* (Banks), is commonly found in rotting cactus tissues in the Sonoran Desert, preying upon a variety of cactophilic insects, especially the cactophilic *Drosophila* (*D. mojavensis*, *D. arizonae*, *D. nigrospiracula*, *D. mettleri*, and *D. pachea*) (Castrezana and Markow, unpublished). Dispersal of *Dinocheirus arizonensis* is facilitated by a behavior termed phoresy in which the pseudoscorpion attaches to the legs of vagile cactophilic insects and is transported to a new host cactus when the insect disperses. Phoretic dispersal is also known for several other members of the order Pseudoscorpiones (Ranius and Douwes, 2002; Moulds et al., 2007; Murienne et al., 2008). The hitchhiking behavior of *D. arizonensis* and its transporter has only been studied in detail on the neriid cactus fly *Odontoloxozus longicornis* (Coquillett), which is also preyed upon by *D. arizonensis* (Zeh and Zeh, 1992), but in the field we have observed *D. arizonensis* attached to cactus beetles (tribe Hololeptini) and to syrphid flies, suggesting that the symbiosis is not specific (Castrezana, unpublished). Phoretic dispersal provides an obvious benefit to *D. arizonensis*, which would otherwise be severely limited in its ability to disperse and colonize new rots. If phoresy is the primary

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mechanism for dispersal in *D. arizonensis* in the wild, and the transporter shows high dispersal capability which is not compromised by the hitchhiker, then we would predict relatively high gene flow and little genetic structure among populations of the pseudoscorpion. Conversely, if phoresy represents only a small fraction of overall dispersal, pseudoscorpion populations should be more highly structured, given their limited capacity to disperse on their own and the patchy nature of their microhabitat. In the present study we examine the population structure and demographic history of the pseudoscorpion *D. arizonensis* collected from 12 localities in the Sonoran Desert using DNA sequence data from a single mitochondrial marker, a segment of cytochrome *c* oxidase subunit I (COI). We also present preliminary results on population structure of the neriid *O. longicornis*, and provide a summary and comparison of the demographic histories of *D. arizonensis*, *O. longicornis* and the cactophilic *Drosophila*.

2. Materials and methods

2.1. Sampling

A total of 91 adult pseudoscorpions was collected during May–June 2002 and January 2008 from necrotic tissue of a variety of columnar cacti (saguaro, cardón, organ pipe, agria and senita); 43 pseudoscorpions were obtained from eight localities on the Baja California (Baja) peninsula and 48 were collected from seven localities on the mainland (Sonora and southern Arizona; Fig. 1; Table 1). The sample included two morphologically distinct species, *D. arizonensis* ($N = 85$) and the cheliferid pseudoscorpion *Parachelifer hubbardi* (Banks) ($N = 6$). Although sample size in *P. hubbardi* was too small for population genetic analyses, the COI sequence data for this species were included in the phylogenetic analyses. A small sample ($N = 28$) of larval and adult neriid flies, *O. longicornis*, was collected along with *D. arizonensis* at three localities on the mainland (Guaymas and San Juanico, Sonora, and Tucson, Arizona); additional specimens of neriids were taken in the Sierra Ancha, Arizona and at San Bruno, Baja California Sur (Fig. 1; Table 1).

2.2. DNA extraction and amplification

Total genomic DNA was extracted from tissue samples using the DNeasy™ (QIAGEN Inc., Valencia, CA) protocol. The polymerase chain reaction (PCR) was used to amplify a segment of the COI gene (~700 bp) with primers LCO1490f (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198r (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) using standard PCR conditions (Folmer et al., 1994). Verifica-

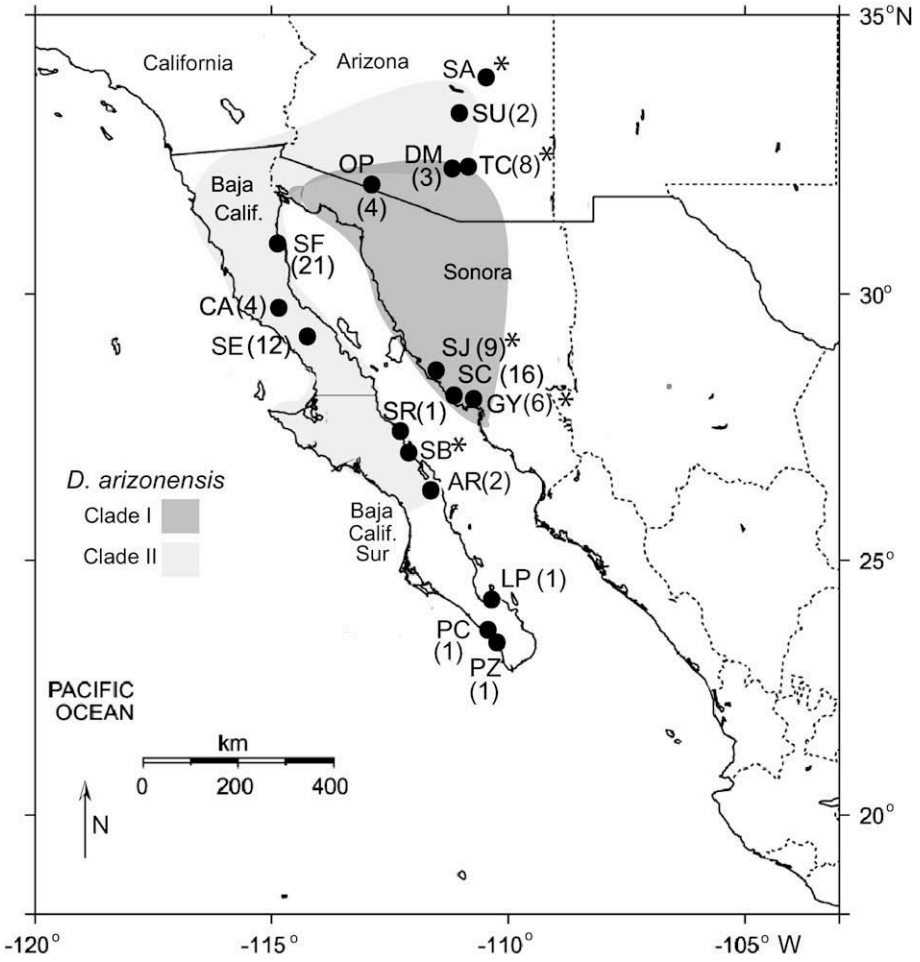

Fig. 1. Map showing collecting localities (black dots) in northwestern Mexico and southwestern USA. Total number of pseudoscorpions (*Dinocheirus arizonensis* and *Parachelifer hubbardi*) collected at each locality is shown in parentheses; asterisks indicate localities where neriid flies *Odontoloxozus longicornis* were taken (see Table 1 for details). Shaded areas represent approximate geographical distributions of *Dinocheirus arizonensis* clade I and clade II inferred from the molecular data. Abbreviations (Arizona): SA, Sierra Ancha; SU, Superstition Mountains; TC, Tucson; DM, Arizona-Sonora Desert Museum; OP, Organ Pipe Cactus National Monument; (Sonora): SJ, San Juanico; SC, San Carlos; GY, Guaymas; (Baja California Sur): LP, La Paz; PZ, Pozo 100; PC, Pozo Cota; AR, Armenta; SB, San Bruno; SR, Santa Rosalía; (Baja California): SE, Sepultura; CA, Cataviña; SF, San Felipe.

Table 1
Summary of the geographic distribution and number of individuals collected of the pseudoscorpions *Dinocheirus arizonensis* and *Parachelifer hubbardi* and the neriid fly *Odontoloxozus longicornis*.

Locality	Abbrev.	*D. arizonensis*		*P. hubbardi*	*O. longicornis*
		Clade I	Clade II		
Arizona					
Sierra Ancha	(SA)				4
Superstition Mts.	(SU)		2		
Tucson	(TC)	2	6		10
Desert Museum	(DM)		2	1	
Organ Pipe NM	(OP)	3		1	
Sonora					
San Juanico	(SJ)	9			3
San Carlos	(SC)	16			
Guaymas	(GY)	6			10
Baja California Sur					
La Paz	(LP)			1	
Pozo 100	(PZ)			1	
Pozo Cota	(PC)			1	
Armenta	(AR)		2		
San Bruno	(SB)				1
Santa Rosalía	(SR)		1		
Baja California					
Sepultura	(SE)		11	1	
Cataviña	(CA)		4		
San Felipe	(SF)		21		
	Total	36	49	6	28

tion of successful amplification was assessed by agarose gel electrophoresis.

Sequencing reactions were performed on an Applied Biosystems (Foster City, CA) ABI 3730XL DNA sequencer at the DNA Sequencing Facility, University of Arizona, using the amplifying primers. Sequences were proofread and aligned in either Sequencher 4.1 (GeneCodes Corp.) or ClustalX 1.81 (Thompson et al., 1997) followed by manual editing. Sequences were trimmed to remove ambiguous sites, resulting in a final segment of 551 bp in the pseudoscorpions *D. arizonensis* and *P. hubbardi* and 639 bp in the neriid fly *O. longicornis*. Aligned sequences were translated in MEGA version 3.1 (Kumar et al., 2004) using the invertebrate mitochondrial genetic code; no stop codons or indels were found. Calculations of genetic distances among sequences [uncorrected *p*-distances and K2P distances (Kimura, 1980)] were carried out in MEGA. Calculations of genetic diversity indices were performed in DnaSP version 4.10 (Rozas et al., 2003). Sequences of all unique COI haplotypes have been deposited in GenBank under the following Accession Nos. *D. arizonensis* (FJ483786–FJ483812), *P. hubbardi* (FJ483813–FJ483816) and *O. longicornis* (FJ532245–FJ532254).

2.3. Population genetic analyses

Hierarchical analysis of molecular variance (AMOVA, Excoffier et al., 1992) performed in ARLEQUIN version 3.1 (Excoffier et al., 2005) was used to test for population structure in *D. arizonensis* for populations with $N \geqslant 3$. For the AMOVA, populations were divided into two groups representing the two clades (I and II) found for *D. arizonensis* (see Section 3.2). The hierarchical AMOVA partitioned genetic variation among localities relative to the total sample (Φ_{ST}), among localities within clades (Φ_{SC}), and among clades I and II (Φ_{CT}). The calculation of significance of the fixation indices Φ_{ST}, Φ_{SC}, and Φ_{CT} was based on 10,000 permutations of the data matrix. The significance of population pairwise comparisons of Φ_{ST} was assessed using a sequential Bonferroni correction for multiple comparisons (Rice, 1989). Pairwise estimates of the number of migrants per generation (N_m) among populations assumed to be in mutation-drift equilibrium were also calculated in ARLEQUIN. We also performed AMOVA on populations of *O. longicornis* from two widely-separated localities, Tucson and Guaymas ($N = 10$ for each), where individuals of *D. arizonensis* were also collected (Table 1).

2.4. Phylogenetic analyses

Relationships among COI haplotypes from the entire pseudoscorpion data set were initially assessed with the neighbor-joining (NJ) algorithm of Saitou and Nei (1987) carried out in MEGA using a matrix of uncorrected *p*-distances. This initial analysis revealed that haplotypes of *D. arizonensis* partitioned into two monophyletic clades as mentioned previously. All analyses of demographic history, therefore, were conducted on each clade separately. Relative rate tests (Tajima, 1993) of sequence evolution in clades I and II of *D. arizonensis* were carried out in MEGA using *P. hubbardi* as the outgroup.

A subset of COI sequences comprised of each of the haplotypes in *D. arizonensis* and *P. hubbardi* were used to conduct phylogenetic analyses using maximum parsimony (MP) and Bayesian inference. The MP analyses were carried in MEGA using the CNI heuristic search option and 100 random additions of sequences. Relative support for tree topology was obtained by bootstrapping (Felsenstein, 1985) using 1000 pseudoreplicates. Bayesian methods were implemented in MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001). The model of nucleotide substitution that best fit the data set, determined with Modeltest 3.7 (Posada and Crandall, 1998) using the Akaike Information Criterion, was GTR + Γ. Bayesian analyses were run under the parameters of this model (nst = "6"; rates = "gamma") for 1,000,000 generations, sampled every 250th generation (4000 trees sampled), using the default random tree option to begin the analysis. Clade support, expressed as posterior probabilities, was estimated utilizing a Markov chain Monte Carlo (MCMC) algorithm. Log-likelihood values from four simultaneous MCMC chains (three hot and one cold) stabilized at about 10,000 generations. The first 40 trees, therefore, were discarded from the analysis (burnin = 40). The cheliferid pseudoscorpion, *Protochelifer naracoortensis* (GenBank Accession No. DQ184919), was used as the outgroup.

2.5. Demographic analyses

Statistical tests designed to assess whether nucleotide polymorphisms deviate from expectations under neutral theory [Tajima's (1989) *D* and Fu's (1997) F_S] were carried out in ARLEQUIN. These tests also are sensitive to factors other than selection, including population expansions and bottlenecks, with Fu's F_S being especially sensitive in detecting population expansions which lead to large negative values in the test statistic (Fu, 1997; Ramos-Onsins and Rozas, 2002). Significance of *D* and F_S values was determined in ARLEQUIN using 1000 simulated samples to produce an expected distribution under selective neutrality and population equilibrium. The cut-off level for statistical significance was 0.05. For Fu's F_S, significance at the 0.05 level was indicated when *P* values were <0.02 (Excoffier et al., 2005).

Significant values for Fu's F_S suggested that both clades of *D. arizonensis* and *O. longicornis* had experienced historical population expansions. Their demographic histories, therefore, were explored further utilizing three different tests of the sequence data: (1) analysis of the distribution of pairwise sequence differences (mismatch distribution; Harpending, 1994) performed in ARLEQUIN; (2) Bayesian skyline analysis implemented in BEAST version 1.2 (Drummond et al., 2005); and (3) estimation of changes in population size carried out in FLUCTUATE version 1.4 (Kuhner et al., 1998).

For populations which have undergone an historical expansion, plots of the distribution of pairwise differences among haplotypes are expected to be unimodal, whereas populations in equilibrium generally show a multimodal distribution (Harpending, 1994). Under the sudden expansion model the parameters generated are τ, the time to the population expansion (=$2ut$, where u is the mutation rate for the entire gene segment and t is the number of generations since the expansion), and the mutation parameters θ_0 and θ_1, where $\theta_0 = 2uN_0$, and $\theta_1 = 2uN_1$ (N_0 and N_1 are the population sizes before and after the expansion, respectively) (Rogers and Harpending, 1992). The significance of the estimated parameters is obtained by calculating the sum of square deviations (SSD) statistic and the raggedness statistic (rg; Harpending, 1994), and their corresponding P values (Excoffier et al., 2005). The sudden expansion model is rejected when $P < 0.05$.

The Bayesian skyline analysis utilizes MCMC sampling of sequence data to estimate a posterior distribution of effective population size through time (Drummond et al., 2005). Bayesian skyline analyses were run under the conditions of the GTR + Γ model (four gamma categories). The mean mutation rate per site per generation (μ) was set at 1.15×10^{-8}. We arrived at this rate by assuming (i) an average pairwise sequence divergence rate of 2.3% per million years (Brower, 1994) and (ii) a generation time of one year. The number of grouped intervals (m) was set to ten. Five million iterations of the MCMC chains were run, sampling every 1000 iterations; the first 500,000 chains were discarded as burnin. The Bayesian skyline plots were generated with TRACER version 1.2.1 (Drummond et al., 2005).

The FLUCTUATE program provides an estimate of long-term female effective population size (N_{ef}) and evaluates whether N_{ef} has changed or remained stable over time (Kuhner et al., 1998). The simultaneous maximum-likelihood estimates of the mutation parameter θ (where $\theta = 2N_{ef}\mu$) and the exponential population growth parameter (g) were obtained from a final extended run of ten short chains of 100,000 steps each and two long chains of 200,000 steps each, sampling every 20th step. Initial estimates of θ were based on number of segregating sites (Watterson, 1975), with the random tree default setting selected for the starting genealogy.

3. Results

3.1. Genetic diversity

Genetic diversity indices for *D. arizonensis*, *P. hubbardi* and *O. longicornis* are shown in Table 2. In *D. arizonensis*, values for both haplotype diversity (h) and nucleotide diversity (π) were greater in clade I than in clade II. For both clades, however, nucleotide diversity was low ($\pi = 0.0044–0.0064$) and haplotype diversity was high ($h = 0.746–0.897$). Overall values for the combined clades in *D. arizonensis* are also given in Table 2 for comparison. Fu's F_S was significant in both individual and combined clades of *D. arizonensis*, as well as in *P. hubbardi* and *O. longicornis*. Tajima's D was significant in both clade I and clade II of *D. arizonensis* and in *O. longicornis*. Relative rates tests (Tajima, 1993) were not significant in *D. arizonensis*, indicating that a molecular clock could not be rejected.

3.2. Phylogenetic relationships

Initial NJ analyses of pseudoscorpion COI sequences (not shown) showed that *P. hubbardi* formed a highly-supported lineage separate from *D. arizonensis*, as expected for two distantly related species from different families. Average genetic distance between the two species was 24.6% (uncorrected p-distance) and 29.9% (K2P distance). The two species also showed 41 fixed amino acid differences in the translated COI gene segment of 183 amino acids. The initial NJ tree also showed *D. arizonensis* resolving as two well-supported clades (clades I and II). Maximum parsimony and Bayesian analyses confirmed the partitioning of clades I and II, but support for the split was weaker, especially in the Bayesian tree (Fig. 2). Clade I was comprised of individuals of *D. arizonensis* from mainland Sonora and southeastern Arizona. Clade II was comprised individuals from the Baja peninsula and southeastern Arizona. Individuals from both clades were found at Tucson (Fig. 1; Table 1). Mean genetic distance (uncorrected p-distance and K2P distance) among individuals of clades I and II was 2.6%. There were nine fixed nucleotide substitutions in the 551 bp gene segment among clades I and II, eight of which were at the third codon position. A single first codon position substitution at site 413 resulted in a valine to isoleucine amino acid change in the COI protein segment of clade I individuals, a substitution also seen in *P. hubbardi* and the outgroup species *Protochelifer naracoortensis*.

3.3. Population structure

The hierarchical AMOVA conducted on combined clade I and II populations of *D. arizonensis* (Table 3) revealed significant structure (overall $\Phi_{ST} = 0.860$; $P < 0.0001$), with 80.23% of the genetic variation distributed between clades I and II ($\Phi_{CT} = 0.802$; $P = 0.026$). Most (13 of 16) of the pairwise comparisons of Φ_{ST} between populations of clades I and II were also significant using a sequential Bonferroni correction (Table 4). Only 5.81% of the genetic variation was distributed among populations within clades, but the corresponding fixation index (Φ_{SC}) was significant ($\Phi_{SC} = 0.294$; $P < 0.0001$). However, only three of the within-clade pairwise comparisons of Φ_{ST} were significant (Table 4). For the most part, the estimated number of migrants per generation (N_m) between populations within each clade was $\geqslant 1.0$, whereas pairwise values of N_m between populations of clades I and II were all low ($\leqslant 0.15$). One exception is the clade I population from Organ Pipe Cactus National Monument (OP) in which the consistently higher within-clade pairwise Φ_{ST} values, and lower N_m values, suggest a pattern of isolation by distance. Overall, the results of the AMOVA, while indicating some within-clade population structuring, suggest a degree of gene flow among these populations consistent with phoretic dispersal. Therefore, all clade I populations and

Table 2
Summary of genetic diversity indices and results of neutrality tests (Tajima's D and Fu's F_S) in the COI gene segment in the pseudoscorpions *Dinocheirus arizonensis* and *Parachelifer hubbardi* and the neriid fly *Odontoloxozus longicornis*.

Species	N	L	k	K	h (±SD)	π (±SD)	Tajima's D	Fu's F_S
D. arizonensis	85	551	52	27	0.898 ± 0.022	0.0161 ± 0.0008	−0.54	−24.65*
Clade I	36	551	26	14	0.897 ± 0.030	0.0064 ± 0.0010	−1.58*	−25.98*
Clade II	49	551	24	13	0.746 ± 0.057	0.0044 ± 0.0008	−1.82*	−26.71*
P. hubbardi	6	551	9	4	0.800 ± 0.172	0.0067 ± 0.0024	−0.42	−2.81*
O. longicornis	28	639	13	10	0.778 ± 0.066	0.0024 ± 0.0005	−1.76*	−27.57*

N, number of sequences; L, sequence length (bp); k, number of variable sites; K, number of haplotypes; h, haplotype diversity; π, nucleotide diversity.
* Significant at the 0.05 level.

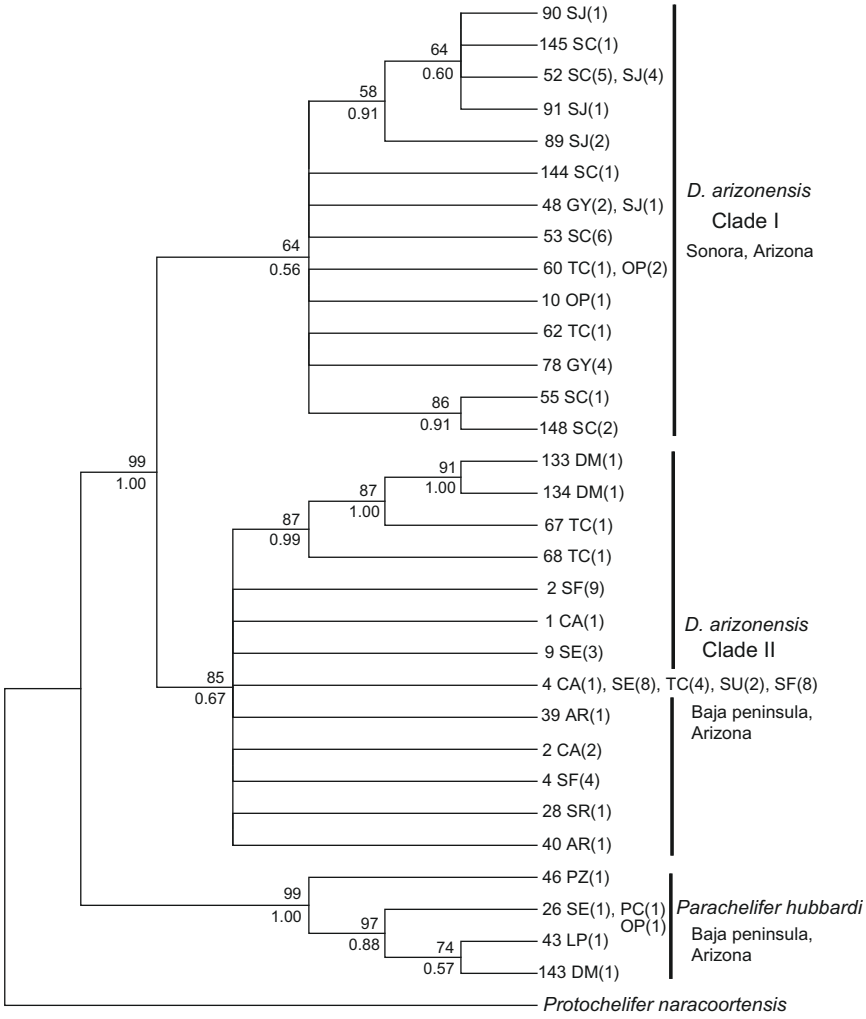

Fig. 2. Most parsimonious tree (length = 233; CI = 0.906; RI = 0.961) obtained using each of the COI haplotypes found in the pseudoscorpions *Dinocheirus arizonensis* (Chernetidae) and *Parachelifer hubbardi* (Cheliferidae) from northwestern Mexico and southeastern Arizona (156 parsimony informative sites). The cheliferid *Protochelifer naracoortensis* was used as the outgroup. Clade support values are shown on branches; nodes with <50% support were collapsed. Bootstrap support values for the maximum parsimony tree are shown above the branches; posterior probability values for the 50% majority rule Bayesian tree are shown below the branches. Branch terminals are labeled with sample identification number and locality abbreviation (see Fig. 1). The number of individuals with the same haplotype at each locality is given in parentheses.

Table 3
Hierarchical analysis of molecular variance (AMOVA) for populations of *Dinocheirus arizonensis* grouped by clade I and clade II.

Source of variation	*df*	Sum of squares	Variance components	% of variation
Among groups	1	229.697	5.93892Va	80.23
Among populations within groups	6	27.772	0.43024 Vb	5.81
Within populations	68	70.242	1.03296 Vc	13.95
Total	75	327.711	7.40212	
Fixation indices				
$\Phi_{ST} = 0.860^{*}$ ($P < 0.0001$)				
$\Phi_{SC} = 0.294^{*}$ ($P < 0.0001$)				
$\Phi_{CT} = 0.802^{*}$ ($P = 0.026$)				

* Significant at the 0.05 level.

all clade II populations were each combined for the tests of demographic history. Results of AMOVA conducted on the populations of *O. longicornis* from Guaymas and Tucson revealed a lack of population structure and high gene flow between these localities ($\Phi_{ST} = 0.004$; $P = 0.579$; $N_m = 122.5$).

3.4. Historical demography

Plots of the distribution of pairwise differences among COI haplotypes in clades I and II of *D. arizonensis* and in *O. longicornis* (Fig. 3) conformed to expectation for populations that have undergone expansions. For both species, the mismatch distribution test statistics SSD and rg were small and not statistically significant at the 0.05 level (Table 5), indicating that the sudden expansion model could not be rejected. The values of τ (time to the population expansion) in Table 5 were used to obtain estimates of *t*, the number of generations since the expansion, using the equation $\tau = 2ut$ and assuming a mean mutation rate per site per generation (μ) of 1.15×10^{-8} (see Section 2.5). The results are shown in Table 6, along with estimates of *t* for several species of cactophilic *Drosophila*, important prey for *Dinocheirus arizonensis* and *O. longicornis*. The results suggest a range of population expansions beginning about 60,000 generations ago (*Drosophila mettleri* from the Baja peninsula) to about 700,000 generations ago (*D. mojavensis* from the mainland). Estimates for the beginning of the population expansions in *Dinocheirus arizonensis* fall within this range (293,000 and 214,000 generations ago for clades I and II, respectively).

Table 4
Pairwise comparisons of Φ_{ST} (below the diagonal) and number of migrants per generation (N_m; above the diagonal) for populations of *Dinocheirus arizonensis*.

	Clade I				Clade II			
	GY (6)	SC (16)	SJ (9)	OP (3)	SE (11)	CA (4)	SF (21)	TC (6)
GY	—	1.79	0.99	0.27	0.04	0.07	0.05	0.09
SC	0.22	—	2.81	0.55	0.08	0.10	0.08	0.11
SJ	0.33*	0.15	—	0.61	0.09	0.13	0.08	0.15
OP	0.65	0.48*	0.45	—	0.04	0.06	0.06	0.11
SE	0.92*	0.86*	0.85*	0.93*	—	1.04	1.27	1.89
CA	0.88	0.83*	0.79*	0.89	0.32	—	0.94	2.67
SF	0.90*	0.87*	0.86*	0.90*	0.28*	0.35	—	1.20
TC	0.84*	0.82*	0.77*	0.82	0.21	0.16	0.29	—

Significant pairwise Φ_{ST} values after a sequential Bonferroni correction ($P < 0.0025$) are indicated with asterisks. Number of individuals from each locality is shown in parentheses. Populations with a sample size <3 [i.e., the clade I population from Tucson and four clade II populations from the Baja peninsula and southern Arizona (Table 1)] were omitted from the analysis. Locality abbreviations are given in Table 1.

Table 5
Results of the mismatch distribution of COI sequences in clades I and II of the pseudoscorpion *Dinocheirus arizonensis* and the neriid fly *Odontoloxozus longicornis*.

Species	τ (95% CI)	θ_0	θ_1	SSD	rg
D. arizonensis					
Clade I	3.71 (1.67, 5.14)	0.000	32.93	0.025 ($P = 0.052$)	0.058 ($P = 0.089$)
Clade II	2.71 (0.00, 5.52)	0.004	3.64	0.022 ($P = 0.32$)	0.074 ($P = 0.44$)
O. longicornis	1.34 (0.38, 2.22)	0.004	>1000	0.017 ($P = 0.15$)	0.114 ($P = 0.14$)

See Section 2.5 for explanation of abbreviations.

Results of analyses of COI sequence data using FLUCTUATE generally were consistent with those of the mismatch distribution. In clade II of *D. arizonensis* and *O. longicornis*, the exponential population growth parameter (g) was positive and significantly different from zero (Table 7), indicating population growth. In clade I of *D. arizonensis*, the value for g was positive but it was not significantly different from zero, or no population growth.

Bayesian skyline plots (Fig. 4), showing the estimated changes in median N_{ef} over time for *D. arizonensis* and *O. longicornis*, were concordant with results from the mismatch distribution and FLUCTUATE. Fig. 4 shows that after a long period of relative population stability, both clades I and II of *D. arizonensis* experienced population expansions dating roughly to similar time periods (~260,000 years before present). The population expansion in *O. longicornis* dates to ~100,000 years before present. These values agree well with the time intervals for both species shown in Table 6, assuming a generation time of one year. The magnitude of the population increases for *D. arizonensis* shown in Fig. 4, however, was greater in clade II than in clade I, in agreement with results from FLUCTUATE (Table 7).

4. Discussion

4.1. Genetic divergence and population structure in Dinocheirus arizonensis

Phylogenetic analyses revealed that populations of *D. arizonensis* within the Sonoran Desert partitioned into two clades (clades I and II) separated by a mean genetic distance (d) of 2.6%. The genetic distance between the two geographically isolated (except in southeastern Arizona) clades, together with the presence of nine fixed nucleotide differences and one fixed amino acid substitution in the COI gene segment, suggest that they represent two distinct evolutionary lineages that have diverged in allopatry. Results from

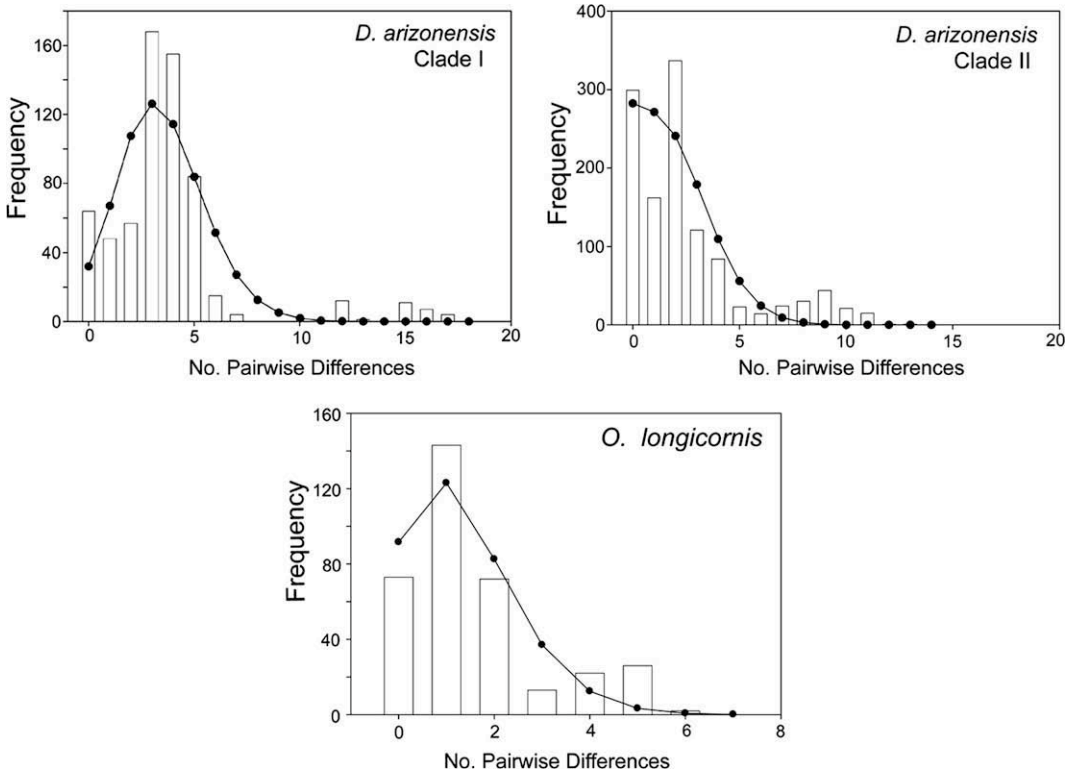

Fig. 3. Distribution of pairwise differences among COI haplotypes (mismatch distribution) in clades I and II of the pseudoscorpion *Dinocheirus arizonensis* and in the neriid fly *Odontoloxozus longicornis* (vertical bars). Solid lines represent the expected distributions under the sudden expansion model. The unimodal distribution of observed pairwise differences expected for populations which have undergone an expansion is seen in each of the plots.

Table 6
Estimates of number of generations since the population expansion (t) in *Dinocheirus arizonensis*, the neriid fly *Odontoloxozus longicornis* and the cactophilic *Drosophila* based on the mismatch distribution.

Species	N	COI (bp)	u	τ (95% CI)	t (generations)
D. arizonensis					
Clade I	36	551	6.34×10^{-6}	3.71 (1.67, 5.14)	2.93×10^{5}
Clade II	49	551	6.34×10^{-6}	2.71 (0.00, 5.52)	2.14×10^{5}
O. longicornis (Baja and mainland)	28	639	7.35×10^{-6}	1.34 (0.38, 2.22)	0.91×10^{5}
Drosophila					
nigrospiracula (Baja and mainland)	94	655	7.53×10^{-6}	3.00 (0.36, 3.50)	1.99×10^{5}
pachea (Baja)	142	661	7.60×10^{-6}	5.39 (1.62, 9.62)	3.55×10^{5}
mettleri (mainland)	45	662	7.61×10^{-6}	1.39 (0.00, 3.68)	0.91×10^{5}
mettleri (Baja)	50	662	7.61×10^{-6}	0.91 (0.46, 1.60)	0.60×10^{5}
mojavensis (mainland)	47	658	7.57×10^{-6}	10.47 (0.47, 64.00)	6.92×10^{5}
mojavensis (Baja)	63	658	7.57×10^{-6}	2.70 (0.00, 5.91)	1.78×10^{5}

Mismatch distribution parameters for the cactophilic *Drosophila* were calculated from the COI data of Hurtado et al. (2004) and Reed et al. (2007). Populations of *Drosophila* that showed significant structure within a region were omitted from the analysis. A mean mutation rate per site per generation (μ) of 1.15×10^{-8} was assumed. The parameter u is the mutation rate for the entire gene segment [i.e. μ times the number of base pairs (bp)]. The parameter t (generations) was calculated from the equation $\tau = 2ut$ (Rogers and Harpending, 1992).

Table 7
Effective female population sizes (N_{ef}) and exponential growth rates (g) in clades I and II of the pseudoscorpion *Dinocheirus arizonensis* and the neriid fly *Odontoloxozus longicornis* calculated with FLUCTUATE.

Species	No. of COI sequences	θ	N_{ef}	g ($1/\mu$ generations)
D. arizonensis				
Clade I	36	0.0167 (±0.0057)	7.26×10^{5}	72 (±99)
Clade II	49	0.0180 (±0.0064)	7.83×10^{5}	271 (±200)
O. longicornis	28	0.0266 (±0.0175)	2.31×10^{6}	1703 (±814)

Values for maximum-likelihood estimates of θ and g (±1.96 standard deviations) are shown. A neutral mutation rate per site per generation (μ) of 1.15×10^{-8} was assumed.

the AMOVA were consistent with this conclusion, indicating that 80% of the genetic variation in *D. arizonensis* was partitioned between the two clades. We attribute the co-occurrence of clades I and II in Arizona to secondary contact after divergence (see below).

Although only a few molecular studies have been conducted on the order Pseudoscorpiones (Murienne et al., 2008), these studies are beginning to reveal a high level of intraspecific genetic differentiation. For example, divergences in the COI gene larger than those seen in *D. arizonensis* have been found among different populations of the cheliferid cave pseudoscorpion *Protochelifer cavernarum* in Australia (Moulds et al., 2007). Also, in the chernetid pseudoscorpion *Cordylochernes scorpioides* from Panama and South America, COI divergences (K2P distances) ranged from 2.6% between populations from Trinidad and French Guiana to 13.8% between populations from Panama and South America (Wilcox et al., 1997). Even within Panama, three highly divergent lineages of *C. scorpiodes* have been reported (Zeh et al., 2003). Most population comparisons of *C. scorpiodes* among regions also showed 1–3 amino acid substitutions in the COI protein segment (Wilcox et al., 1997), consistent with the differences seen in clades I and II of *D. arizonensis*.

Because a molecular clock could not be rejected for sequence evolution in *D. arizonensis*, the mean genetic distance between clades I and II can be used to estimate dates of when the two clades began to diverge. As with the chernetid pseudoscorpion *C. scorpioides*, however, a calibrated molecular clock is not available for *Dinocheirus*. Thus, we have applied the commonly used standard rate of 2.3% pairwise sequence divergence per million years for mitochondrial DNA in arthropods (Brower, 1994), a rate also used for *C. scorpiodes* (Zeh et al., 2003). Because several lines of evidence suggest that the mitochondrial COI gene in some arthropods evolves at a slower rate of ~0.6–1.5% pairwise sequence divergence per million years (Farrell, 2001; Pfeiler et al., 2006) we have also estimated dates using an average rate of 1.0%. The mean sequence divergence of 2.6% found among clades I and II suggests that the two began to split from a common ancestor roughly 1.1 million years ago (Ma) during the mid Pleistocene using the 2.3% clock, or 2.6 Ma during the mid-to-late Pliocene using the 1.0% clock. The temporal framework provided by the molecular clocks suggests two scenarios that may have led to disruption of gene flow in the ancestral population of *D. arizonensis*. Marine incursions of the Gulf of California into southeastern California and southwestern Arizona occurred during the late Miocene and early Pliocene (McDougall et al., 1999; Riddle et al., 2000; Oskin and Stock, 2003) forming a potential barrier to dispersal of terrestrial organisms on either side of the seaway. The ability of the neriid fly *O. longicornis* and other vagile insects to transport pseudoscorpions over expanses of water by phoresy is unknown, but it seems highly probable that dispersal of the pseudoscorpions would be diminished in the presence of such a barrier. Even in the absence of a water barrier, Plio-Pleistocene climate transitions and associated glacial cycles would probably have affected populations of both the pseudoscorpions and their aerial transporters, potentially resulting in reduced phoretic dispersal and increased reproductive isolation between mainland and peninsular populations of the pseudoscorpions. By the end of the Pleistocene, when climatic conditions became more stable and the northern Gulf had receded to its present position, the disjunct populations could have come into secondary contact, explaining the sympatric occurrence of clades I and II seen today in southeastern Arizona.

We predicted that if phoresy plays an important role in dispersal and colonization of patchily distributed resources in *D. arizonensis*, populations of the non-vagile pseudoscorpion should show little structure throughout its range in the Sonoran Desert. When clades I and II are analyzed separately, our results generally support this conclusion, with populations showing relatively high genetic connectivity within each of the divergent clades. The prediction obviously assumes high genetic connectivity of the host transporter over the same geographic range as the pseudoscorpion, an assumption supported by our preliminary results on *O. longicornis*. The high gene flow seen in *O. longicornis* from the mainland Sonoran Desert is also in agreement with results obtained for most species of cactophilic *Drosophila* (Pfeiler and Markow, 2001; Markow et al., 2002; Hurtado et al., 2004; Ross and Markow, 2006; Reed et al., 2007).

Although samples of *D. arizonensis* used in the present study were collected from several species of columnar cacti, we found no evidence for genetic differentiation resulting from cactus host type. For example, high gene flow was seen in clade II populations from the Baja peninsula, where samples were obtained from senita,

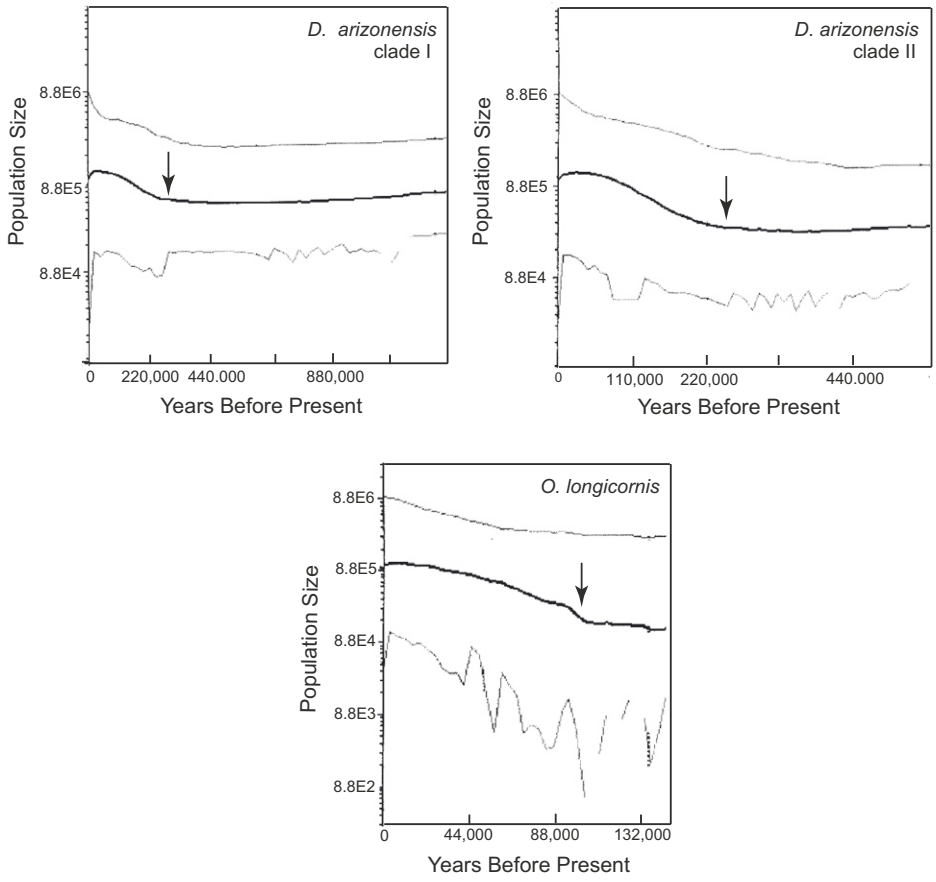

Fig. 4. Bayesian skyline plots showing changes in effective female population size (N_{ef}) over time for clades I and II of the pseudoscorpion *Dinocheirus arizonensis* and for the neriid fly *Odontoloxozus longicornis*. Population size is given on a logarithmic scale. A value of 1.15×10^{-8} for μ, the mean mutation rate per site per generation, was assumed. The thick solid lines represent the median estimates of population size; the thin solid lines show the 95% HPD (highest posterior density) intervals. Note the different time scales used in the three plots. Arrows show estimated dates for the beginning of the population expansions in clades I and II of *Dinocheirus arizonensis* (~260,000 years before present) and in *Odontoloxozus longicornis* (~100,000 years before present).

agria, organ pipe and cardón, to southeastern Arizona where samples were obtained from saguaro.

4.2. Demographic history of Dinocheirus arizonensis

Results of different tests of demographic history were generally congruent and suggested that both clades I and II of *D. arizonensis* have experienced similar historical population expansions. As described earlier, we have assumed a standard 2.3% molecular clock and a generation time of one year in the tests of demographic history. Generation times of *D. arizonensis* in the wild, however, are not known, but it is highly probable that more than one generation is produced each year. Laboratory experiments have shown that the period of development from fertilization to adult is about 2–3 months in *D. arizonensis* (Zeh, 1987). In addition, females are known to store sperm for extended periods and produce more than one brood from a single mating (Zeh and Zeh, 1992). Because of the various assumptions associated with the estimation of μ, the mean mutation rate per site per generation for *D. arizonensis*, the time axes in the Bayesian skyline plots (Fig. 4) should be considered only rough estimates. But regardless of the number of generations per year, and assuming it is the same in clades I and II, it is apparent that each clade began to increase its population size at about the same time (Table 6; Fig. 4).

Results of different tests of demographic history in *O. longicornis* also were generally congruent and suggested that it too has undergone an historical population expansion, similar to the expansions found for the cactophilic *Drosophila* which utilize the same necrotic microhabitat and serve as prey for *O. longicornis* and *D. arizonensis* (Hurtado et al., 2004; Machado et al., 2007; Pfeiler et al., 2007). The estimates of number of generations since the population expansion (Table 6) suggest that in the cactophilic *Drosophila* (with the exception of *D. mettleri*) the population expansions roughly coincide with those seen in clade I and II of *D. arizonensis* of ~200,000–300,000 generations ago. The population expansion in *O. longicornis* began approximately 90,000 generations ago, the same as in *Drosophila mettleri* from the mainland. We must emphasize that the values of t shown in Table 6 are only rough estimates given the large confidence intervals surrounding the values of τ and the fact that we assumed the same COI mutation rate and a one year generation time for all species. Nonetheless, the historical increases seen in population size of *D. arizonensis*, *O. longicornis* and the cactophilic *Drosophila* suggest a complex interaction and ecological balance among predator, prey and phoretic dispersal of *D. arizonensis*.

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