MSCP vernal pool inventory
City of San Diego (USFWS)
Conservation genetics of the endangered fairy shrimp species Branchinecta sandiegonensis

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This is the final report for the contract "*Genetic testing of the endangered fairy shrimp species* Branchinecta sandiegonensis" to Andrew J. Bohonak from the City of San Diego. This contract was set up in late 2002 and work for the project officially began January 1, 2003. The contract ended in June 2005. Marie A. Simovich (University of San Diego), a subcontractee and full collaborator on this project. Simovich is permitted by USFWS for work on *B. sandiegonensis*, and Bohonak is listed under that permit.

A scientific publication based on the data summarized here will be submitted for publication. A copy of this manuscript will be provided to Keith Greer (City of San Diego) and Jonathan Snapp-Cook (U.S. Fish and Wildlife Service).

Summary

A genetic study based on mtDNA sequencing of *B. sandiegonensis* from across its range found two evolutionary significant units "ESUs" that should be strongly considered for unique conservation status. Pool complexes that are in undisturbed areas are often genetically unique.

Motivation

Worldwide changes in land use (primarily agriculture and urbanization) have led to a global loss of temporary wetlands. In southern California, it is estimated that 95% of the vernal pools have been lost (Bauder 1998 and references therein). The threats to these naturally fragmented habitats are compounded by their inherent natural isolation at both local and regional scales. (Local metapopulations of ponds are found in areas where hydrologic conditions facilitate pool formation. Regionally, these pool complexes are separated kilometers or tens of kilometers by geologically unfavorable terrain.) Contemporary and historical connectivity between pools at these two scales is largely a matter of speculation (Bohonak & Jenkins, 2003). Because the continued loss of vernal pools may interact in complex ways with future climate change, there are many uncertainties concerning the persistence of vernal pool habitats, their associated ecosystem services and the endangered species they maintain (Pyke 2004).

Fairy shrimp (Crustacea: Branchiopoda: Anostraca) are relatively large crustacean zooplankton (> 10 mm) restricted almost entirely to temporary wetlands worldwide. At least five Anostracan species are listed on the U.S. Endangered Species list, with more under consideration. Over 30 fairy shrimp are considered threatened or endangered worldwide. This project examined population genetic structure in the federally endangered fairy shrimp *Branchinecta sandiegonensis* in order to gain insight into contemporary and historical connectivity among pools and pool complexes, and make conservation recommendations.

Prior to this study, only allozymes had been used to study genetic structure in this species (Davies et al. 1997), and there are very few DNA-level population genetic studies for any fairy shrimp. Davies et al. (1997) found significant genetic differentiation among 10 pools for *B. sandiegonensis* using allozymes, and evidence for a "temporal Wahlund effect" within pools. (The importance of overlapping generations created by the fairy shrimp cyst bank may be reflected in heterozygote deficiencies within each pond.) The goal of this study was to expand coverage to include the majority of the species range, including all pool complexes on City of San Diego property. The choice of mitochondrial DNA sequence variation over allozymes for this study reflects the higher degree of precision that can be obtained with mtDNA sequencing. Also, sequence-level variation permits a wider range of analyses that can be used to separate contemporary and historical processes such as allopatric isolation and gene flow.

Methods

Fairy shrimp were collected as adults or cysts, or hatched from sediment samples by Marie A. Simovich. Individuals were collected from across southern California, identified to species according to Eriksen and Belk (1999), and stored in 95% ethanol or at -80° C until analysis. A map containing the collection locations (City and non-City) is provided in Figure 1. We chose to sample additional ponds not specifically located on City of San Diego property, so that our results represent the dispersal biology and evolutionary history of this species across its entire range. These additional samples were analyzed using funds obtained by Bohonak and Simovich from other sources.

Protocol for amplifying a 658 bp portion of the mitochondrial gene cytochrome oxidase I (COI) was adapted from existing lab protocol for arthropods. (Bohonak has developed universal primers similar to LCO-1490 and HCO-2198 of Folmer et al. 1994). PCR products were cycle sequenced using BigDye v. 3 termination (Perkin-Elmer) and sequenced on an ABI 377 automated sequencer or and ABI 3100 sequencer. Sequence alignments were conducted by eye using the program Sequencher. (Alignment is largely trivial, since COI is a protein-coding gene, and no insertions or deletions were detected.) Some individuals were cycle sequenced in both directions.

Evolutionary relationships among haplotypes were determined using maximum parsimony with PAUP 4.0 (Swofford 2001), and with network parsimony reconstruction as implemented in TCS (Clement et al. 2000). General population genetics summary statistics were calculated using PAUP.

Results

General summary statistics

DNA was analyzed from 316 individual *B. sandiegonensis* from 75 pools in 30 "pool complexes". (A pool complex is a local metapopulation of hydrologically linked pools). An additional 31 fairy shrimp from other species (*B. coloradensis, B. lynchi, B. lindahli*) were also sequenced for use in comparative studies and as outgroups. From the 316 *B. sandiegonensis* sequenced, 50 unique haplotypes ("alleles") have been found. (Each allele is a sequence that differs from all other alleles by one or more base pairs.) The average divergence between all

alleles is 1.65%, and the maximum divergence is 3.04%. Of 657 bp sequenced from the CO I gene, 478 are constant, 132 are parsimony informative, and 47 are parsimony uninformative.

Haplotype distributions

Table 1 summarizes allele distributions within and among pools, pool complexes and geographic regions. (Note: Although the City's original labeling scheme called geographic regions "Complexes" and local metapopulation of hydrologically connected pools "Sites", I refer to a local hydrologically linked set of pools a "complex" in this report.) For clarity, Table 1 lists pools nested within complexes, nested within regions.

There are two dominant features in this data set. First, the numbers generally fall out along a diagonal, indicating that pool complexes are often fixed for unique haplotypes found nowhere else in the species. There is a high degree of endemicity apparent within local groups of hydrologically linked pools, and genetic differentiation among regions is high. This is particularly obvious in areas such as Ramona, Otay Mesa, Otay Lakes and Marron Valley, which have less influence from development and recreation than sites in Mira Mesa and Del Mar.

Second, two groups of haplotypes can be distinguished: "A" and "B". Alleles within group A or B differ from each other by relatively few mutational differences (avg. 0.78% divergence, maximum 1.52%). Divergence between A and B is much more pronounced (avg. 2.52% between pairs of alleles, maximum 3.04%). This indicates that individuals from Group A and B have been isolated from one another biologically for many thousands (or perhaps millions) of years with little or no dispersal or hybridization.

Pools in the Nobel, Mira Mesa, Del Mar and the Montgomery Field/General Dynamics areas tend to have more alleles than pools in areas that are relatively pristine (e.g., Otay Lakes, Marron Valley, Ramona, Murphy Canyon: see Table 1).

Geographic and phylogenetic analysis

A maximum parsimony analysis was conducted with PAUP (100 bootstraps, heuristic search), using 6 sequences from *B. lynchi, B. lindahli* and *B. coloradensis* as outgroups. The consensus tree and bootstrap values are presented in Figure 2. *B. sandiegonensis* is monophyletic in 100% of the bootstraps, supporting the designation of this species as it is currently recognized. Clades A and B are monophyletic 91% and 92% of the time, respectively, indicating that these are also likely to be real evolutionary units. The sister species to *B. sandiegonensis* cannot be determined with this particular analysis; it is unclear whether the addition of more genes or the use of a model-based analysis (e.g., Bayesian estimation of the phylogeny) would be needed to resolve this question.

Specific conclusions

These analyses indicate that:

1) *B. sandiegonensis* represents a monophyletic taxon (i.e., a "good species" from an evolutionary perspective) in this data set. It is monophyletic in 100% of the bootstraps conducted. Additional genetic and morphological analyses of the genus will be needed to resolve additional taxonomic issues. I recommend maintaining the current nomenclature at this time.

2) There is considerable genetic variation within this species.

3) There is high mtDNA divergence among vernal pool "complexes" that are, in some cases, only tens of kilometers apart.

4) It is obvious that gene flow between pool complexes is lower in areas that are less impacted by development and recreation (e.g., vernal pool complexes in Ramona, Otay Mesa, Otay Lakes and Marron Valley). The simplest conclusion is that human activities tend to artificially homogenize natural populations of *B. sandiegonensis* and increase (rather than decrease) the genetic variation in any particular pool. Consequently, local adaptation to the unique hydrological, biological and chemical aspects of each pool complex may be hindered in these areas.

5) There is a deep split between clades "A" and "B". The clades are reciprocally monophyletic on most trees.

6) Clades A and B have unusual allopatric distributions (outlined in Figure 1), which do not correspond to any known current or past geologic features.

7) Only 4 individual fairy shrimp of 316 analyzed violate the generalized distributions of clade A and B in Figure 1. (Note the outlying "1"s in Table 1.) These appear to represent very recent introductions of shrimp:
   a) from {Nobel Drive, Del Mar, Mira Mesa or Carmel Mountain} into {Ramona}
   b) from {Nobel Drive, Del Mar, or Mira Mesa} into {Sander}
   c) from {Marron Valley} into {Mission Trails}
   We recommend that all vernal pool researchers and consultants thoroughly clean their boots and nets after visiting each site.

8) There is some phylogenetic structure within clades A and B that indicates long-standing geographic isolation. For example, haplotypes A16 and A26, restricted to the border region, form a monophyletic group. The same is true for:
   {A17, A18, A23} restricted to Marron Valley and Otay Mesa,
   {A19, A20, A21, A22} restricted to Del Mar,
   {B5, B17} found only in Ramona and Pendleton
   {B21, B14, B15, B16}. B21 is found only San Onofre; the others are restricted to Miramar.

9) Haplotype A25, found only in Costa Mesa, is most similar to haplotypes found in Otay Mesa, possibly indicating a long distance dispersal event.

10) Evolutionary significant units "ESUs" that should be considered for conservation include the two major clades (A and B) and many individual pool complexes. According to some interpretations of the ESU concept, every pool complex that is genetically unique could be considered an ESU worthy of separate consideration. Full scientific acceptance of this would likely require additional genetic analyses with other markers and studies demonstrating morphological, physiological and/or ecological divergence as well.

Caveats

The taxonomic status of *Branchinecta sandiegonensis* is outside the scope of this study.

Literature cited

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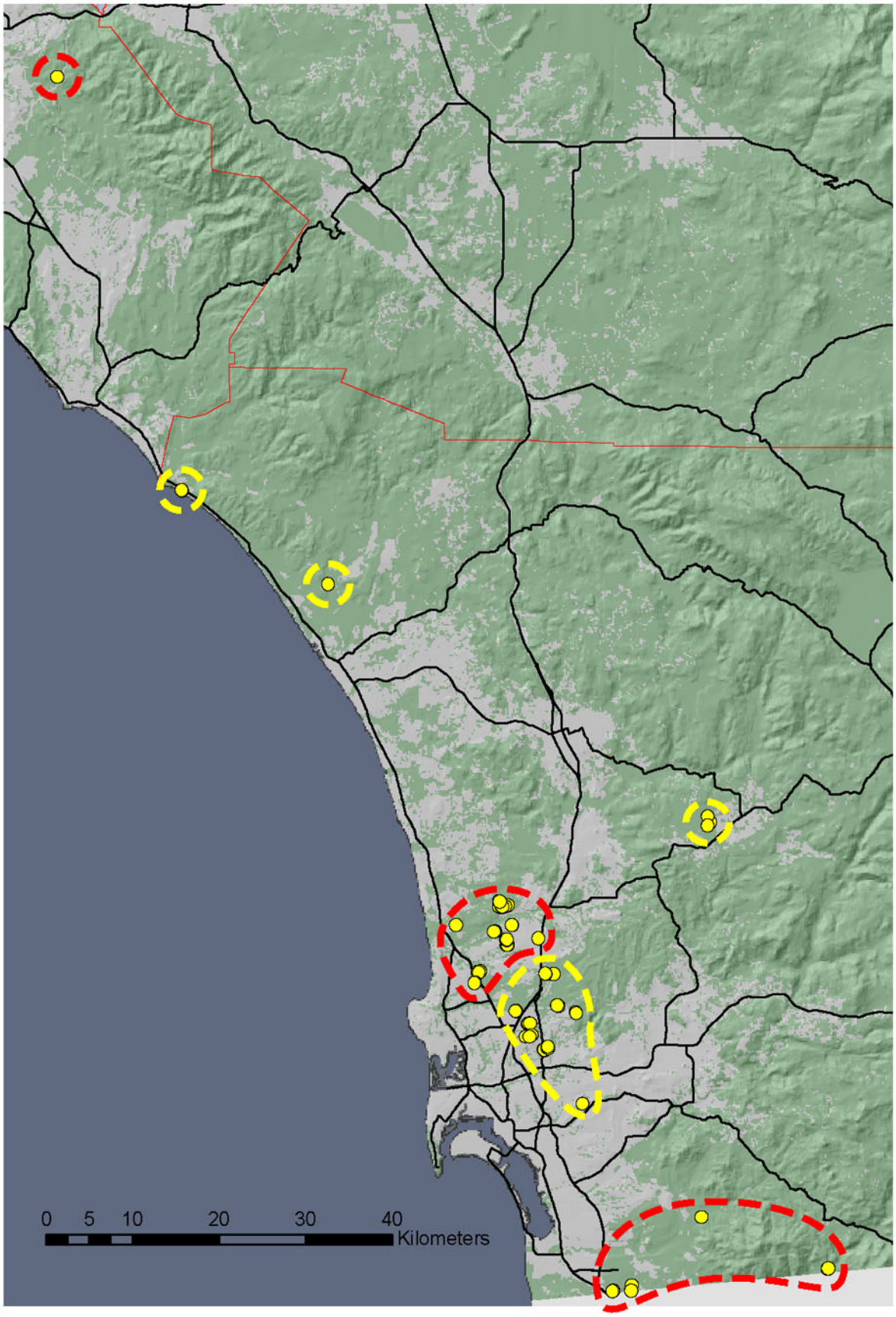
Table 1: Haplotype distributions within and among pool "complexes" (local metapopulations of hydrologically linked pools).

Figure 1: Geographic distribution of samples, with the primary distributions of clades A and B circled in red (clade A) and yellow (clade B). Only 4 individuals violate these primary distributions (see Table 1). Yellow dots indicate ponds sampled, with many overlapping points. The location of the sample from Pendleton is approximate.

Figure 2: mtDNA gene tree for *Branchinecta sandiegonensis*. (Maximum parsimony tree, 50% majority consensus, nodes indicate bootstrap support).

			Haplotype																																					
Region	Complex	Pond	A07	A08	A10	A01	A02	A21	A22	A12	A14	A20	A11	A04	A19	A13	A15	A06	A03	A29	A05	A25	A16	A26	A09	A24	A27	A18	A23	A28	A17									
Nobel drive	Nobel drive	1	1	1		1	7																																	
		2					4																																	
		3			1		2																																	
	Eastgate	1				1		1		1																														
		2					4																																	
		4?					2																																	
Del Mar	Bowtie	1				1		2	1	1																														
		2						2	2																															
		3						1		2	1																													
	Del Mar Mesa North	1				1		1				1																												
		2				1		1																																
		3				2		2																																
	Del Mar Mesa East	2					1			1																														
		3								1																														
		4											3	1																										
		5								2			1	1																										
		RR1				1		1	1	1					1																									
Mira Mesa	Cousins	1				5																																		
	Mesa Verde	1					4																																	
		2				1	3																																	
		3				5																																		
	Winterwood	1				3										2																								
		2					1			1			1																											
		3				1											1																							
		4								2																														
		5				2										1		2																						
	Brown	1				1							2																											
		2				3																																		
		3				1							1						2																					
	Maddox	1				17														1	1																		
		2				3				1																														
		4				3																																		
		7				1															3																		
Carmel Mountain	Carmel Mountain	1				5																																		
Costa Mesa	Costa Mesa	D																				1	2																
Otay Lakes	Otay Lakes	1																					7	1															
Otay Mesa	Snake Cholla	1																						2															
		2																					5		1														
	Arnie's Point	1																								3	2												
		2																										2	1										
	J16-18	1																								1		1		2									
	Goat Mesa	2																										5											
Marron Valley	Marron Valley	3																													5								
		5																													5								
Ramona	Ramona	7				1																																		
		17B																																						
		W6																																						
Pendleton	DZ Tank Park	DZTP																																						
Miramar	AA10	68.3E																																						
		MC5																																						
		MC6																																						
	AA9	K4																																						
		MC4																																						
	A4	103.5																																						
		105																																						
		MC9																																						
Mission Trails	Mission Trails	1																																						
		3																																						1
MFGD	General Dynamics	1																																						
		2																																						
		3																																						
	Sander	1																																						
		2					1																																	
		4					1																																	
		5																																						
	Montgomery Field	3																																						
		5																																						
		6																																						
Chollas	Chollas	2																																						
Murphy Canyon	Murphy Canyon	A2																																						
		A5																																						
		B1																																						
		B3																																						
San Onofre	San Onofre	A																																						
		C																																						
Grand Total			1	1	1	60	30	11	4	13	1	1	8	2	1	3	1	2	2	1	4	1	14	3	1	4	2	8	1	2	11								

Region	Complex	Pond	B05	B17	B15	B14	B16	B01	B06	B03	B07	B02	B13	B10	B11	B18	B19	B04	B20	B09	B12	B08	B21	Grand Total
Nobel drive	Nobel drive	1																						10
		2																						4
		3																						3
	Eastgate	1																						3
		2																						4
		4?																						2
Del Mar	Bowtie	1																						5
		2																						4
		3																						4
	Del Mar Mesa North	1																						3
		2																						2
		3																						4
	Del Mar Mesa East	2																						2
		3																						1
		4																						4
		5																						4
		RR1																						5
Mira Mesa	Cousins	1																						5
	Mesa Verde	1																						4
		2																						4
		3																						5
	Winterwood	1																						5
		2																						3
		3																						2
		4																						2
		5																						5
	Brown	1																						3
		2																						3
		3																						4
	Maddox	1																						19
		2																						4
		4																						3
		7																						4
Carmel Mountain	Carmel Mountain	1																						5
Costa Mesa	Costa Mesa	D																						3
Otay Lakes	Otay Lakes	1																						8
Otay Mesa	Snake Cholla	1																						2
		2																						6
	Arnie's Point	1																						5
		2																						3
	J16-18	1																						4
	Goat Mesa	2																						5
Marron Valley	Marron Valley	3																						5
		5																						5
Ramona	Ramona	7	6																					7
		17B	5																					5
		W6	6	3																				9
Pendleton	DZ Tank Park	DZTP		1																				1
Miramar	AA10	68.3E			2	2	1																	5
		MC5			2	2																		4
		MC6			2	2	1																	5
	AA9	K4			1			3																4
		MC4			5																			5
	A4	103.5						4	1															5
		105						4		1														5
		MC9						2																2
Mission Trails	Mission Trails	1						3			2													5
		3									2	3												6
MFGD	General Dynamics	1						1	3															4
		2							2				1	1	1									5
		3						1								3	1							5
	Sander	1						1										4						5
		2						2										2						5
		4													1									2
		5																3	1					4
	Montgomery Field	3							1															1
		5							5															5
		6							2											1	1			4
Chollas	Chollas	2							6													2		8
Murphy Canyon	Murphy Canyon	A2							2															2
		A5							1															1
		B1							1											1				2
		B3							2															2
San Onofre	San Onofre	A																					2	2
		C																					1	1
Grand Total			17	4	12	6	2	21	26	1	4	3	1	1	2	3	1	9	1	2	1	2	3	316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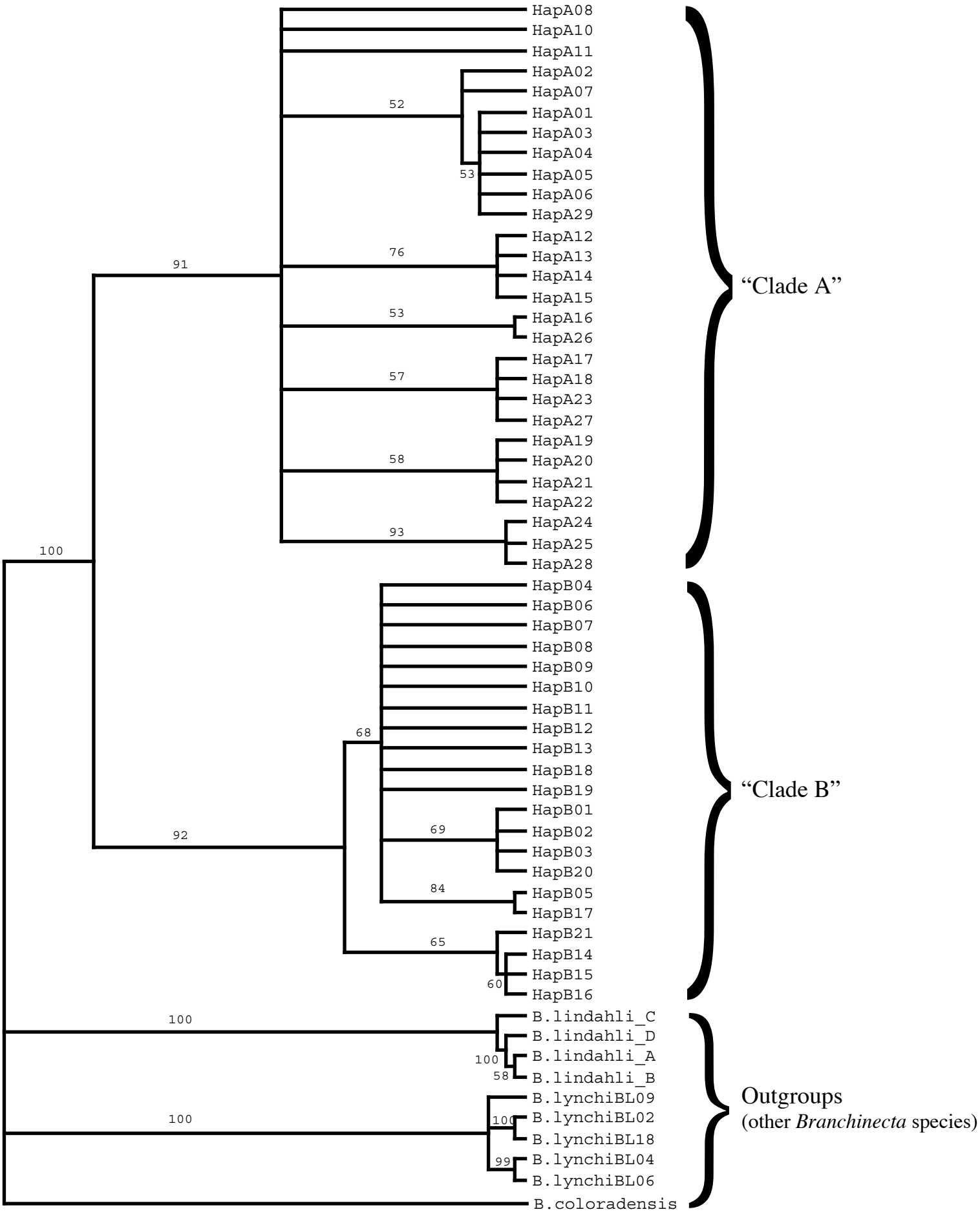
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76
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57
58
93
100
68
92
69
84
65
60
100
100
58
100
100
99
"Clade A"
"Clade B"
Outgroups
(other *Branchinecta* species)