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A clash of characters: The effect of variation on a morphological hybrid index for an endangered California fairy shrimp *Branchinecta sandiegonensis* (Fugate, 1993) (Crustacea: Anostraca)

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ABSTRACT

We validated the morphological hybrid index (MHI) described by [Simovich *et al.* \(2013\)](#) for detecting hybrids between the endangered San Diego fairy shrimp *Branchinecta sandiegonensis* ([Fugate, 1993](#)) and the versatile fairy shrimp *B. lindahli* ([Packard, 1883](#)) through morphological and genetic analysis of 662 individuals. This index uses species-specific character states for “spines” (dorsolateral projections) on thoracic segments 3–11 in adult females. In non-admixed (non-hybrid) populations, the character states for segment 8 correlate poorly with the remaining segments, and the overall morphological hybrid index score (MHI score). Only 22% of non-admixed *B. sandiegonensis* display the expected character state for segment 8 compared to nearly all *B. lindahli*. Eliminating this character in principal component analyses increases the proportion of variation explained by PC1 from 57.3% to 69.5%. The revised MHI, however, still shows slight variation in populations of *B. sandiegonensis* and *B. lindahli* that have no genetic evidence of admixture. Hybrid populations possess considerably more variation in MHI score, and this variation is heavily skewed towards *B. lindahli* phenotypes. We suggest using the revised MHI and genetic characters in future experimental field studies to improve an understanding of the processes that initiate and maintain hybridization in this system.

Key Words: conservation, Pearson product-moment correlation, principal components analysis

INTRODUCTION

The San Diego fairy shrimp *Branchinecta sandiegonensis* ([Fugate, 1993](#)) was listed as a species of concern in 1997 ([U.S. Fish and Wildlife Service, 1997](#)) due to habitat destruction. Only approximately 10% of its original estimated habitat, coastal vernal pools, remains intact ([Bauder & McMillan, 1998](#)). Despite existing only in a fraction of its historic distribution, the San Diego fairy shrimp is the typical species of *Branchinecta* found in coastal vernal pools in southern California, presumably due to its intolerance of more alkaline inland waters ([Gonzalez *et al.*, 1996](#)). [Fugate \(1993\)](#), [Erickson & Belk \(1999\)](#), and [Simovich *et al.* \(2013\)](#) have nevertheless documented *B. sandiegonensis* cohabiting with the versatile fairy shrimp *Branchinecta lindahli* ([Packard, 1883](#)) in some disturbed pools. The versatile fairy shrimp is a geographically widespread generalist that is known for its ability to tolerate a wide range of

pool conditions ([Eng *et al.*, 1990](#); [Aguilar *et al.*, 2017](#)). These species can be differentiated by male secondary antennae ([Fugate, 1993](#); [Erickson & Belk, 1999](#)) and spine morphology on female thoracic segments ([Rodgers, 2002](#)). Genetic analyses using allozymes ([Fugate, 1992](#)) and mitochondrial sequence data ([Bohonak, 2005](#); [Vandergast *et al.*, 2009](#)) have also shown that *B. sandiegonensis* and *B. lindahli* are genetically distinct species. More recent work has shown that *B. sandiegonensis* can hybridize with *B. lindahli* in laboratory conditions and in nature ([Erickson & Belk, 1999](#); [Simovich *et al.*, 2013](#); C. Shanney, R. Clark & A. J. Bohonak, unpublished data). The ability to accurately identify hybrids is therefore paramount to study interspecific hybridization between *B. lindahli* and *B. sandiegonensis* and to the conservation and recovery of the endemic and endangered *B. sandiegonensis*.

[Simovich *et al.* \(2013\)](#) published a morphological hybrid index that distinguishes adult female *B. sandiegonensis*, *B. lindahli*, and

putative hybrids based on the arrangement of spines displayed on each thoracic segment (Fig. 1). This morphological hybrid index provides a cost-effective method to identify hybrids in natural populations and has also served as a framework to develop a genomic hybrid index (GHI) (Patel et al., 2017). The correlation between both indices dissociated in disturbed localities was characterized by significant genetic admixture when morphological hybrid index scores were compared to genomic hybrid index scores in 24 localities (Patel et al., 2017). This finding suggests that abiotic disturbances or introgressive hybridization could contribute to variation in spine morphology of genetically non-admixed and admixed individuals, respectively. Conflict between morphological and genomic hybrid indices motivates a re-examination of the morphological hybrid index of Simovich et al. (2013) in terms of intraspecific and interspecific morphological variation. We analyzed the robustness of spine morphology on each thoracic segment as a metric for morphologically detecting hybrids, using individuals of known genomic ancestry.

METHODS

A subset of the total ($N = 663$) 561 genetically non-admixed *Branchinecta* females were used to test the robustness of spine morphology on thoracic segments as metrics to distinguish *B. sandiegogenesisis* from *B. lindahli* (Supplementary material Table S1). Adult females were scored morphologically as described in Simovich et al. (2013). The body of each specimen was rotated so that spine morphology could be observed on multiple sides. Spine morphology on each of the nine segments was characterized based on Simovich et al. (2013), and scores for each thoracic segment were averaged to generate a composite morphological hybrid score (MHI score) for each specimen (Fig. 2). Individuals were classified morphologically as *B. lindahli* (MHI score = 1–1.39), admixed (MHI score = 1.4–2.59), and non-admixed *B. sandiegogenesisis* (MHI score = 2.6–3.0) based on morphological score.

We quantified the correlation between thoracic segment scores with the MHI score of individuals using pairwise Pearson Product Moment correlations (Flegel et al., 1999) implemented in R (R Core Team, 2013). We found in preliminary analyses that segment 8 had the lowest correlation with the MHIs as well as with all other segments (Table 1). To further quantify the departure of segment 8 from correlations with all other segments and the MHI score, we performed principal components analyses (PCAs) using 561 individuals from 44 populations with no evidence of genetic admixture using the genomic hybrid index developed by Patel et al. (2017). The genomic hybrid index is comprised of 20 single nucleotide polymorphism (SNP) loci that display fixed allelic differences between *B. sandiegogenesisis* and *B. lindahli* (e.g. “T/T” in *B. sandiegogenesisis*, “C/C” in *B. lindahli*, and “T/C” in F_1 hybrids). Non-admixed populations should therefore display genomic hybrid index scores (GHI score) fixed for either *B. lindahli* (e.g. GHI score = 1) or *B. sandiegogenesisis* (GHI score = 3). We analyzed this dataset of non-admixed populations using data from all nine segments, and again after excluding segment 8. Principal component (PC) scores (Jackson, 1993), implemented in R (R Core Team, 2013), were compared to quantify the influence of data from segment 8 on interspecific morphological variation.

We finally analyzed morphology in a more inclusive dataset of 662 individuals that also included 12 admixed populations (Supplementary material Tables S1, S2, and S3). Segment 8 was not included in the MHI scores for this analysis.

RESULTS AND DISCUSSION

Genetically “pure” *B. sandiegogenesisis* (GHI score = 3) showed the most variation at segment 8, with only 22% of individuals showing expected character states (i.e. two dorsolateral spines). In addition, only half of the individuals displayed typical character states (i.e. two dorsolateral spines) for segment 9 (Table 3). There was

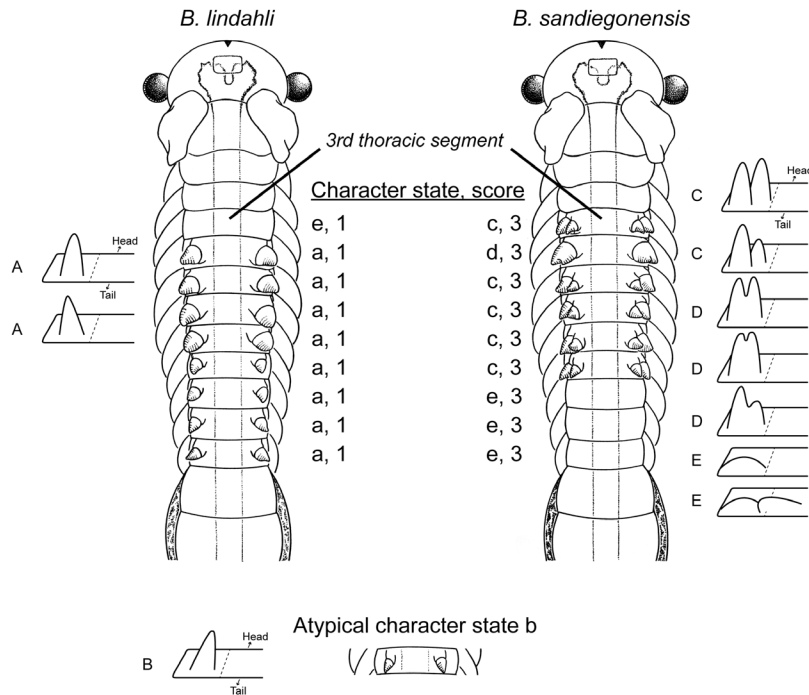


Figure 1. Typical and atypical thoracic spine patterns in *Branchinecta lindahli* and *B. sandiegogenesisis*. Segment 8 and segment 9 can also display only a single dorsolateral spine (modified from Simovich et al., 2013).

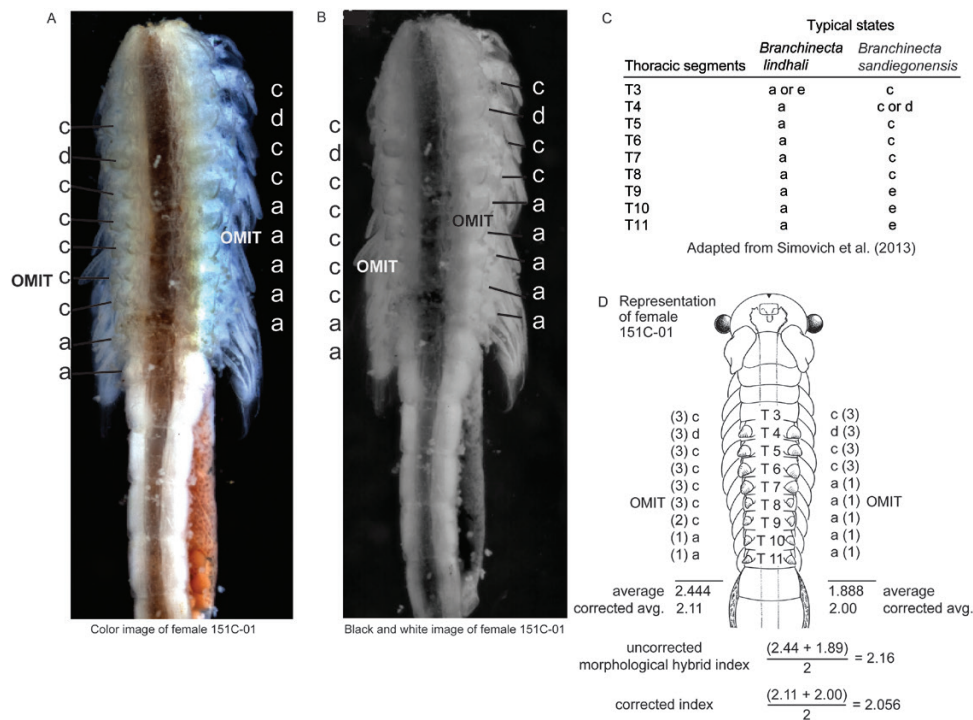


Figure 2. Workflow demonstrating the process of assigning MHI scores to adult females. A high contrast image of an adult female shrimp using a Cannon EOS 5D single-lens reflex camera (SRL), using a 65 mm lens at 2× magnification, mounted on a Visionary Digital BK + imaging system. A series of images were combined into a composite, and the contrast was enhanced using Adobe Photoshop CS3 to better visualize the characters (A). The same image was also shown without color (B). Expected character states and MHI scores for non-admixed individuals (Simovich et al., 2013) (C). A stylized representation of the imaged specimen with spinal patterns (Erikson & Belk, 1999; Simovich et al., 2013), alphabetical character and MHI scores, and calculation of a composite hybrid score. The score in black is the original MHI score; the corrected score is the new score after excluding segment (D).

Table 1. Pairwise correlations matrix comparing correlation strength for each thoracic segment as well as composite morphological hybrid index values including segment 8 (MHI score +8) and excluding segment 8 (MHI score -8).

Thoracic segments	T3	T4	T5	T6	T7	T8	T9	T10	T11	MHI score + 8
AT4	0.895	1.000								
AT5	0.884	0.974	1.000							
AT6	0.856	0.941	0.938	1.000						
AT7	0.816	0.890	0.912	0.931	1.000					
AT8	0.276	0.284	0.298	0.322	0.335	1.000				
AT9	0.420	0.495	0.512	0.483	0.473	0.278	1.000			
AT10	0.712	0.786	0.809	0.785	0.760	0.168	0.586	1.000		
AT11	0.765	0.826	0.850	0.828	0.795	0.242	0.510	0.880	1.000	
MHI score +8	0.874	0.937	0.949	0.937	0.916	0.454	0.658	0.864	0.890	1.000
MHI score -8	0.884	0.950	0.961	0.944	0.919		0.654	0.893	0.907	0.988

little or no variation in any segment in non-admixed populations of *B. lindahli* (Table 3). Indeed, illustrations of *B. sandiegonensis* in its original description showed spines on segment 8 that were much smaller than those on segments 5–7 (Fugate, 1993). This difference in size was nevertheless not included in the schematic diagrams of Erickson & Belk (1999). Consistent with initial characterization of *B. sandiegonensis* by Fugate (1993), we believe that segment 8 represents a transitional point in morphology between dual spines or fused spines in segments 3–7, and no spines in segments 9–11. We found that segment 8 in non-admixed *B. sandiegonensis* can display double spines (22% of individuals), single spines (38%), or a combination of double, single, or no spines on opposing sides (17%) (Fig. 1).

Correlations among individual thoracic segments and the MHIs ranged from 0.66–0.95 when segment 8 was included and 0.65–0.96 when segment 8 data was excluded (Table 1). When segment 8 was removed, the correlation strength increased for all segments except 9, which decreased slightly from 0.66 to 0.65. More importantly, the proportion of variation explained by PC1 increased from 57.3% to 69.5% when segment 8 was removed (Table 2).

Given our findings, we excluded data from thoracic segment 8 in all subsequent analyses. We found no *B. lindahli* from genetically “pure” populations with MHI scores above 1.3 but 21 of 528 (4.0%) *B. sandiegonensis* from genetically pure populations had MHI scores below 2.6. Even after excluding segment 8, slight morphological variation thus exists in populations of *B. sandiegonensis* with

little or no genetic admixture (Table 3; Fig. 3). The range of morphological variation in these 40 populations is limited to 2.375–3, with nearly all individuals scoring between 2.8–3 (Supplementary material Table S1). We nevertheless found that in non-admixed *B. lindahli* populations ($N = 4$) all but two individuals had MHI scores of 1 (Supplementary material Table S2).

Table 2. The first and second principal component scores for non-admixed: “pure” populations of *Branchinecta lindahli* and *B. sandiegonensis* with all segment data, and excluding segment 8.

Thoracic Segments	All segment data		No segment 8 data	
	PC1	PC2	PC1	PC2
T3	-0.338	-0.116	-0.347	-0.215
T4	-0.339	-0.105	-0.348	-0.138
T5	-0.339	-0.099	-0.347	-0.132
T6	-0.346	-0.077	-0.353	-0.155
T7	-0.352	-0.062	-0.357	-0.170
T8	-0.213	0.923		
T9	-0.347	0.220	-0.347	0.922
T10	-0.358	-0.192	-0.374	0.020
T11	-0.344	-0.139	-0.355	-0.122
Proportion of variance	0.573	0.192	0.695	0.182

The 12 genetically admixed populations we surveyed exhibited considerable variation in morphology (Supplementary material Table S3). Patel et al. (2017) found that interspecific hybrids appear to persist beyond the F_1 generation, with most individuals genetically similar to *B. lindahli* (Patel et al., 2017). Morphological variation shows similar patterns; in admixed populations, MHI scores range from 1 to 3 with 28/44 genetically admixed individuals (64%) scoring as typical *B. lindahli* (Fig. 3, Supplementary material Table S1). This skewed distribution in MHI score can be due to at least four causes. The first cause could be attributed to sampling bias, which is unlikely because admixed localities were geographically disparate. Moreover, specimens were haphazardly sampled in all localities so that discriminating individuals based on spine morphology was minimized. The second cause can be attributed to assortative mating based on spine patterns, which could disproportionately produce offspring that resemble *B. lindahli*; C. Shanney, R. Clark & A. J. Bohonak (unpublished data) have shown that *B. lindahli* will readily mate with *B. sandiegonensis* and most intermediate hybrids found in extensively admixed localities resemble *B. lindahli*. A third cause could be that admixed pools could have been founded with a higher frequency of *B. lindahli* than *B. sandiegonensis*. This is quite possible because genetically non-admixed individuals of *B. lindahli* were present in half of admixed localities sampled and are commonly found in disturbed habitats elsewhere (Aguilar et al., 2017). Admixed individuals from surveyed localities likely experienced introgressive hybridization with genetically “pure” *B. lindahli* (Seehausen, 2004). A fourth cause could be that natural selection in hybrid pools favors individuals with higher

Table 3. The percentage of *Branchinecta sandiegonensis* and *B. lindahli* individuals that displayed expected character states in genetically non-admixed populations and the percentage of individuals in admixed populations that display typical *B. sandiegonensis*, typical *B. lindahli*, or atypical scores.

Thoracic segments	<i>B. sandiegonensis</i> (N = 528)	<i>B. lindahli</i> (N = 33)	Admixed N = 46)		
			<i>B. sandiegonensis</i>	<i>B. lindahli</i>	Atypical
3	88.5%	100%	28.6%	56.1%	15.3%
4	97.9%	100%	41.8%	50.0%	8.2%
5	98.1%	100%	42.9%	51.0%	6.1%
6	98.1%	100%	38.8%	52.0%	9.2%
7	96.2%	100%	38.8%	54.1%	7.1%
8	22.1%	100%	14.3%	73.5%	12.2%
9	50.2%	100%	20.4%	61.2%	18.4%
10	92.8%	97%	22.4%	68.4%	9.2%
11	96.2%	97%	22.4%	68.4%	9.2%

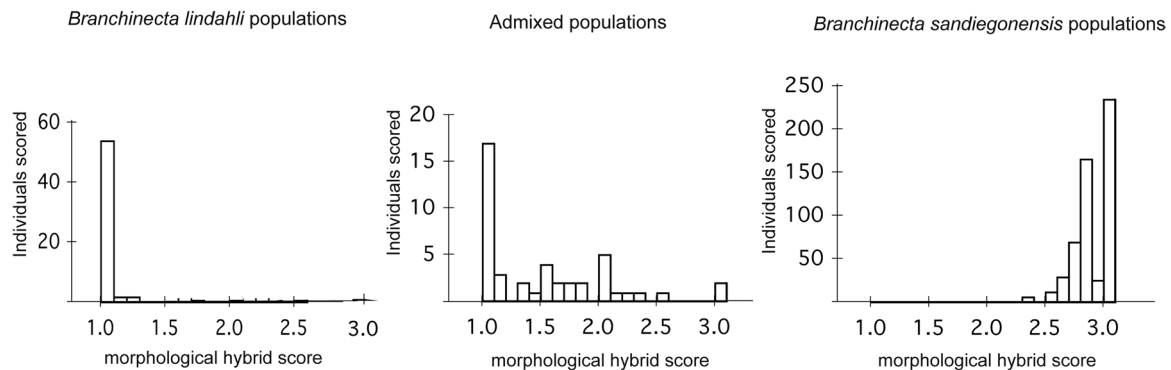


Figure 3. Range in morphology found in non-admixed populations of *Branchinecta lindahli*, admixed, and non-admixed *B. sandiegonensis*, excluding segment 8. Values for non-admixed *B. lindahli* center around 1 with very little skew and the highest score of around 1.25. Morphological variation in admixed populations show that most specimens scores are 1, with a significant number of individuals scoring between 1 and 2.5; scores of 3 can also occur but are rare. Morphological variation in non-admixed *B. sandiegonensis* pools are limited to 2.3–3 with the majority being 2.8–3. Individuals can score less than 2.6 but never below 2.

B. lindahli genomic content, which is also possible because admixed individuals were sampled from severely disturbed localities (e.g. vehicular road ruts and artificial deep impoundments) resembling abiotic conditions similar to inland playas (Simovich *et al.*, 2013). Furthermore, because *B. lindahli* can tolerate a wide variety of environmental conditions, admixed individuals that are genetically and phenotypically more similar to *B. lindahli* than to *B. sandiegonensis* could be more fit because disturbed habitats resemble inland playas more than coastal vernal pools (Simovich *et al.*, 2013).

Because of their cost efficacy and ability to serve as a metric to assess overall genetic makeup, morphological characters are commonly used, as conservation tools, to detect admixed individuals and identify hybrid populations in nature. These characters are most useful when their state variation shows consistency within each species and complete divergence among species. Because spine morphology on thoracic segment 8 correlates poorly with all other segments and genetic determination of the species, we advise that the data from thoracic segment 8 be excluded from the morphological hybrid index of Simovich *et al.* (2013). We also find that a low frequency of morphological variation present in genetically non-admixed populations, due to very low levels of background hybridization or to inherent character variation. Populations with high levels of hybridization tend to resemble *B. lindahli* more than in *B. sandiegonensis*, possibly due to founder effects and greater fitness in disturbed localities. We suggest that an improved understanding of the processes that initiate and maintain hybridization in this system will require the use of this revised female morphological hybrid index to initially detect hybridization and subsequent genetic hybrid index to characterize hybridization in natural populations if present.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Non-admixed localities for *Branchinecta sandiegonensis*.

S2 Table. Non-admixed localities for *Branchinecta lindahli*.

S3 Table. Admixed localities for *Branchinecta sandiegonensis* and *Branchinecta lindahli* hybrids.

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REFERENCES

- Aguilar, A., Maeda-Martínez, A.M., Murugan, G., Obregón-Barboza, H., Rogers, D.C., McClintock, K. & Krumm, J.L. 2017. High intraspecific genetic divergence in the versatile fairy shrimp *Branchinecta lindahli* with a comment on cryptic species in the genus *Branchinecta* (Crustacea: Anostraca). *Hydrobiologia*, **801**: 59–69.
- Bauder, E.T. & McMillan, S. 1998. Current distribution and historical extent of vernal pools in southern California and northern Baja California, Mexico, pp. 56–70. In: Ecology, conservation, and management of vernal pool ecosystems – Proceedings from a 1996 Conference, California Native Plant Society, Sacramento, CA., 1998 (C.W. Witham, E.T. Bauder, D. Belk, W.R. Ferren Jr. & R. Ornduff, eds).
- Bohonak, A.J. 2005. *Genetic testing of the endangered fairy shrimp species Branchinecta sandiegonensis*. Vernal Pool Inventory, City of San Diego and US Fish and Wildlife Service. August 12, 2005 [<https://www.sandiego.gov/planning/programs/mscp/biomonitor/vpi>].
- Eng, L.L., D. Belk & Eriksen, C.H. 1990. Californian Anostraca: distribution, habitat, and status. *Journal of Crustacean Biology*, **10**: 247–277.
- Erickson, C.H. & Belk, D. 1999. *Fairy shrimps of California's puddles, pools, and playas*. Mad River Press, Eureka, CA, USA.
- Fugate, M.L. 1992. *Speciation in the fairy shrimp genus Branchinecta*. Ph.D. thesis, University of California, Riverside, CA, USA.
- Fugate, M.L. 1993. *Branchinecta sandiegonensis*, a new species of fairy shrimp (Crustacea: Anostraca) from western North America. *Proceedings-Biological Society of Washington*, **106**: 296–296.
- Flegel, T.W., Thamavit, V., Pasharawipas, T., & Alday-Sanz, V. 1999. Statistical correlation between severity of hepatopancreatic parvovirus infection and stunting of farmed black tiger shrimp (*Penaeus monodon*). *Aquaculture*, **174**: 197–206.
- Gonzalez, R.J., Drazen, J., Hathaway, S.A., Bauer, B. & Simovich, M.A. 1996. Physiological correlates of water chemistry requirements in fairy shrimps (Anostraca) from southern California. *Journal of Crustacean Biology*, **16**: 315–322.
- Packard Jr, A.S. 1883. Packard's Phyllopod Crustacea. (Letters to the Editor: A Monograph of the Phyllopod Crustacea of North America, with Remarks on the Order Phyllocarida). *Science*, **2**: 571–573.
- Patel, K.V., Bohonak, A.J., Simovich, M.A., Goddard, N.S. & Graige, N.S. 2017. Discovery and validation of species-specific diagnostic SNP markers for the endangered San Diego fairy shrimp (*Branchinecta sandiegonensis*) and the versatile fairy shrimp (*Branchinecta lindahli*). *Conservation Genetics Resources* [doi.org/10.1007/s12686-017-0912-0].
- Rogers, D.C. 2002. Female-based characters for anostracan (Crustacea: Branchiopoda) identification: a key for species of California and Oregon, USA. *Hydrobiologia*, **486**: 125–132.
- Simovich, M.A., Davis, K.B., & Bohonak, A.J. 2013. Landscape homogenization threatens the genetic integrity of the endangered San Diego fairy shrimp *Branchinecta sandiegonensis* (Branchiopoda: Anostraca). *Journal of Crustacean Biology*, **33**: 730–740.
- Vandergast, A.G., Wood, D.A., Simovich, M.A., & Bohonak, A.J. 2009. Species identification of co-occurring *Branchinecta* fairy shrimp from encysted embryos using multiplex polymerase chain reaction. *Molecular Ecology Resources*, **9**: 767–770.
- U.S. Fish and Wildlife Service. 1997. *Endangered and threatened wildlife and plants; determination of endangered status for the San Diego fairy shrimp Federal Register Number 972578*, pp. 4925–4939.