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ORIGINAL ARTICLE

Daphnia lumholtzi Sars, 1885 (Cladocera: Daphniidae) invades Argentina

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ABSTRACT

The extent of freshwater biological invasions is difficult to predict. The thermophilic Daphnia lumholtzi *Sars, 1885 (Cladocera: Daphniidae) has successfully invaded a large section of temperate North America from an Old World source, damaging ecosystems and fisheries. The species was later reported introduced into Mexico and Brazil. Here we report* D. lumholtzi *in Argentina - the most southerly record in the New World for this species. Our genetic analyses establish haplotype identity with North American specimens, consistent with the colonization of South America from North America. The detection dates of the records in South America and the association with the Paraná River, provide evidence that river ways play a role in expansion of* D. lumholtzi*. The invasion of* D. lumholtzi *has now reached a similar wide latitudinal span to the distribution in the Old World.*

Key words: Daphnia, invasion, Cladocera, zooplankton, neotropical.

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INTRODUCTION

Intercontinental invasions are among the most important threats to fragile freshwater ecosystems. However, predicting the geographic extent and success of these invasions has proved difficult (Moyle and Marchetti, 2006; Hayes and Barry 2008). Often the predictive traits are limited to a species, a particular habitat-species combination or a particular stage of invasion. The lack of generalities and predictability for biological introductions has been given pessimistic names such as the Frankenstein effect (Moyle, 1986) and the key-lock effect (Heger and Trepl, 2003).

Cladocerans are one freshwater group that might be expected to have numerous widespread invaders because of their mixed sexual/asexual breeding system - one propagule can lead to successful colonization. However, there is wide variation in the success of cladoceran invaders. *Daphnia obtusa* Kurz, 1874, for example, is known to invade one location in Australia (Benzie and Hodges, 1996). The strictly asexual panarctic *D. pulex*, however, has expanded recently to nearly every continent from its source in North America (Mergeay *et al.*, 2005; Crease *et al.*, 2012; Burns 2013). Other invaders have taken several decades to expand across one continent such as *Bosmina* (*Eubosmina*) *coregoni* Baird, 1857 in North America (Lieder, 1991; Haney and Taylor, 2003). The success of other cladoceran invasions can be difficult to quantify because of hybridization, morphologically challenging taxa, and introductions that occurred before taxonomic discovery.

One of the most morphologically conspicuous invaders among daphniids is *Daphnia lumholtzi* Sars, 1885 in North America. The species is thermophilic, initially distributed predominantly in tropics-subtropics of Old World (Fig. 1A). Large defensive spines and pronounced success in reservoirs in North America have meant that this species has a negative effect on native ecosystems and fisheries (Swaffar and O'Brien, 1996; Havens *et al.*, 2000). *D. lumholtzi* was first established in North America in 1983 in association with mass stocking of African Blue Tilapia *Oreochromis aureus* (Steindachner, 1864) or/and Nile perch *Lates niloticus* (Linnaeus, 1758) to Fairfield Reservoir, Texas (Sorensen and Sterner, 1992; Havel and Hebert, 1993). Then, *D. lumholtzi* rapidly colonized 20 other states, reaching 46-47°N (NW corner of Lake Superior, also present in vicinities of Lake Michigan and Lake Erie), and 27°N moving South (Florida) (Benson *et al.*, 2014). During last decade it was detected in NE Mexico, reaching initially 28-29°N (Elías-Gutiérrez *et al.*, 2008) and then 24°N (Silva-Briano *et al.*, 2010). Dispersal is thought to occur by river systems and by boat traffic. In 2000, *D. lumholtzi* was found in São Paulo State of Brazil, reaching 21°S (Zanata *et al.*, 2003), and then it occupied several water bodies in the upper Paraná River basin in Brazil (Simões *et al.*, 2009), at 22-23°S (Fig. 1B). However, it is still unknown if these introductions are an expansion of the North American invasion or a separate introduction from the Old World tropics. Analysing samples from a survey collected in Argentina in 2006, we found that *D. lumholtzi* occurred much further south in the New World because we found it in Santa Fe Province, at almost 32°S. Our genetic analyses are consistent with expansion of *D. lumholtzi* into southern South America from North America.

METHODS

Collections were made in Argentina and Chile in 2006. *D. lumholtzi* was collected in a pool (coordinates: 31.6631°S; 60.5935°W) adjacent to the Paraná River, between Paraná and Santa Fe, Santa Fe Province on 19 February 2006 by A. Anderson, L. Hovind, W.H. Piel (Fig. 1). Samples were taken by a plankton net and immediately fixed in 96% alcohol. In the laboratory, the samples were sorted under a binocular stereoscopic microscope. Specimens were placed on slides (in a drop of a glycerol-formaldehyde mixture) and studied under an optical microscope *in toto*. Photomicrographs were taken with an Infinity camera attached to the Olympus CX-41 Microscope. DNA extraction from individual ethanol-preserved animals was achieved using the DNA Quickextract extraction kit from Epicentre. Fifty microlitres of PCRs contained 5 µL of extracted DNA template, 10x PCR buffer [50 mM KCl, 1.5 mg MgCl2, 10 mM Tris-HCl, pH 8.3, 0.01% (w/v) gelatin], 2 mM of each dNTP, 1 mM of each primer and 1 U of Taq DNA polymerase. We used primers from Folmer *et al.* (Folmer *et al.*, 1994) for the mitochondrial COI fragment. The PCR temperature profiles were: 40 cycles of 94°C for 30 s, 48°C for 30 s and 72°C for 2 min, and final extension at 72°C for 5 min. DNA was sequenced by Sanger methods at the University of Washington High Throughput Genomics Facility. Geneious 7.0.4 (Biomatters Development Team) was used to assemble, edit and verify open-reading frames. We used MAFFT to align the sequences, jmodeltest 2 Darriba *et al.*, 2012 to assess sequence models, and PhyML (as implemented in Seaview) to estimate best trees under the ML criterion. We used the best tree of NNI (nearest neighbor interchange) and SPR (subtree pruning and redrafting) searches combined. Branch support values were estimated from approximate likelihood ratios (aLRTs). The optimal substitution model as estimated by jModeltest 2 was the HKY 85 (Hasegawa *et al.*, 1985)+gamma.

In addition to our sequences from the specimens collected in Argentina and taken from the Genbank, we obtained new sequences from specimens isolated from the USA and from Thailand (Tab. 1).

RESULTS

The unmistakable morphology of the anterior projection on head, fornices, spinulation on postero-ventral margin of valve etc. in our study reveal that just *D. lumholtzi* has been found (Fig. 2 A-D).

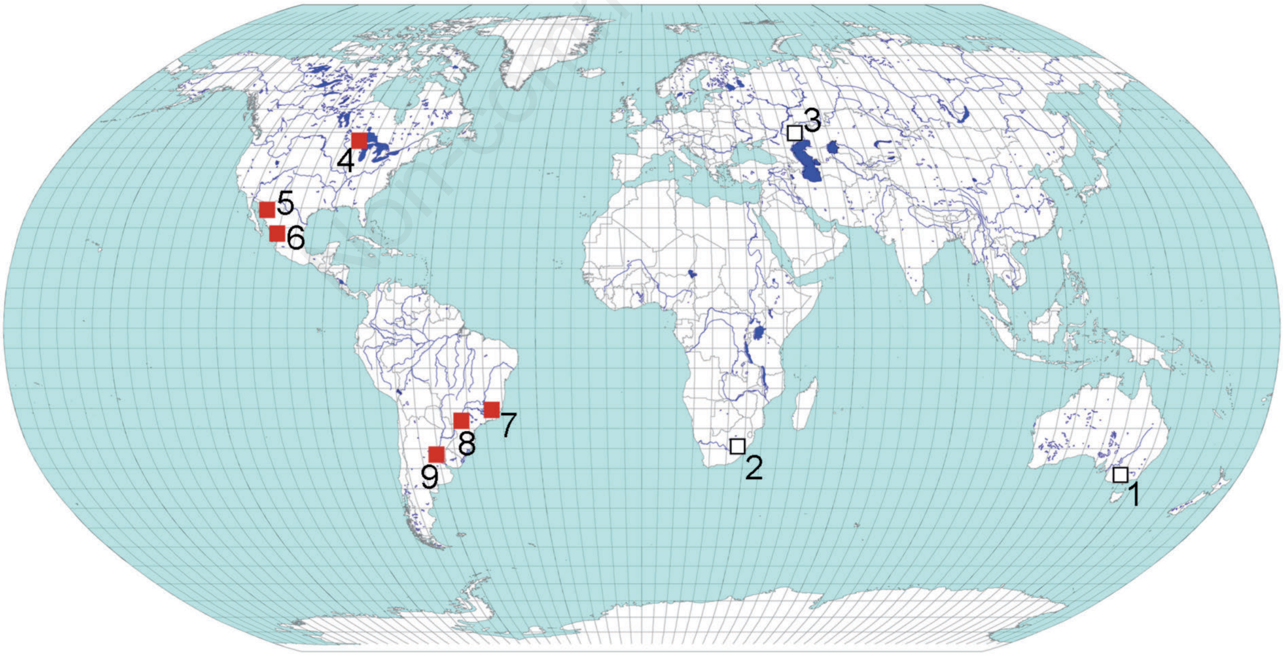

Fig. 1. Some native (empty squares) and invasive (solid squares, red in the electronic version) distributional records of *Daphnia lumholtzi*. Legend to numbers: 1, southernmost point in Australia (Benzie, 2005); 2, southernmost point in Africa (Smirnov, 2008); 3, nothernmost point in Eurasia (Behning, 1928; Glagolev, 1995); 4, northernmost point in North America (Benson *et al.*, 2013); 5, first record in Mexico (Elías-Gutiérrez *et al.*, 2008); 6, southernmost point in Mexico (Silva-Briano *et al.*, 2010); 7, first record in South America (Zanata *et al.*, 2003); 8, next record in Brazil (Simões *et al.*, 2009); 9, this study.

A maximum likelihood tree based on mitochondrial sequence is represented in Fig. 2E. Australian and Asian-African clades of *D. lumholtzi* are deeply divergent and represent at least two independent species. All North American and newly created South American populations definitively belong just to the Asian-African clade [most probably, to an African sub-clade (Havel *et al.*, 2000)]. Among three haplotypes found in Argentina, two are exactly identical to those from USA, and the third one also grouped within the North American clade.

DISCUSSION

The sequence identity of South American specimens with North American specimens is consistent with shared recent ancestry. Australian and Africa-Asian clades form at least two divergent lineages. Their possible differences in biology are unknown. Morphologically cryptic species have been proposed for *D. lumholtzi* in prior studies (Behning, 1928; Kořínek, 2002).

Since *D. lumholtzi* is known to disperse by river systems, the simplest hypothesis for the immediate source of the Argentinian *D. lumholtzi* is dispersal from the Brazilian populations of the Upper Paraná River detected in 2003 (Simões *et al.*, 2009). We found *D. lumholtzi* in the Lower Paraná three years later in 2006. Many water bodies near this large river are frequently flooded and connected with the main river stream during the wet season. Ephippia and parthenogenetic females can disperse by the river current. We suspect that the whole basin of the Paraná River is invaded by *D. lumholtzi* now. Absence of records in the areas between points, where it is found to date, might be explained by insufficient sampling effort, especially in the shallow oxbows near the rivers. *D. lumholtzi* has reached 31-32°S in South America. Therefore its invasive distributional area is significantly increased since the first record on the South American continent (Zanata *et al.*, 2003). High-resolution genetic markers might provide further testing of the origination of the Argentinian populations from Brazilian populations (Frisch *et al.*, 2013). Also mysterious is the method of dispersal from North America to South America. Tropical lowlands (including South America) are relatively poor of *Daphnia* species (Dumont, 1980; Fernando *et al.*, 1987; Paggi, 1993; Popova and Kotov, 2013). Dzialowski *et al.* (2000) hypothesized that by occupying a niche that was previously unexploited by *Daphnia, D. lumholtzi* competed with non-daphnid zooplankton otherwise able to obtain resources during that time. Water bodies lacking aboriginal *Daphnia* species are even more common in tropical-subtropical areas of South America. At the same time, members of the *D. laevis* species group inhabit many tropical-subtropical water bodies of Argentina (Adamowicz *et al.*, 2004). Although lacking projecting fornices, these native daphniids are about the same size as the exotic species, have long caudal needle and high and/or pointed helmet (Herbst, 1967; Paggi, 1977), that protects them against predators in the same way as in *D. lumholtzi*.

Often the original distribution of *D. lumholtzi* is portrayed as tropical or subtropical (Benzie, 2005; Wittmann *et al.*, 2013). However, in the Old World, *D. lumholtzi* s. lat. is distributed North until about 46-47°N (the Volga and Ural deltas, Caspian Region of Russia, see Behning, 1928; Glagolev, 1995). At the same time, in its non-indigenous range in the New World, D. *lumholtzi,* reached almost the same latitudes (about 45°N) as in the Old World. In both South Africa and Australia *D. lumholtzi* is distributed up to (or almost up to) the southernmost extremities of these continents. It was reported from about

Tab. 1. COI gene accession numbers in the GenBank, localities and abbreviations in the tree of studied populations of *D. lumholtzi.*

Abbreviation	Country	Locality	COI gene accession number	Reference
Australia_1	Australia	New South Wales	AF308970.1	Hebert *et al.*, 2002
Australia_2	Australia	New South Wales	AY921417.1	Colbourne *et al.*, 2006
Thailand_1	Thailand	Pond near Bung Pueng, Kalasin Province		This study
USA_MS1	USA	Mississippi	AF308974.1	Hebert *et al.*, 2002
USA_KS1a-b	USA	Keith Sebelius Lake, Kansas		This study
USA_KS2a-d	USA	Wellington Lake, Kansas		This study
USA_NM1a-b	USA	Burn Lake, New Mexico		This study
USA_AZ1a-b	USA	Theodore Roosevelt Lake, Arizona		This study
Mexico_1a-e	Mexico	El Novillo, State of Sonora	EU702127.1, EU702131.1, EU702128.1, EU702129.1, EU702130.1	Elías-Gutiérrez *et al.*, 2008
Argentina_1a-c	Argentina	Pond between Paraná and Santa Fe, Santa Fe Province		This study

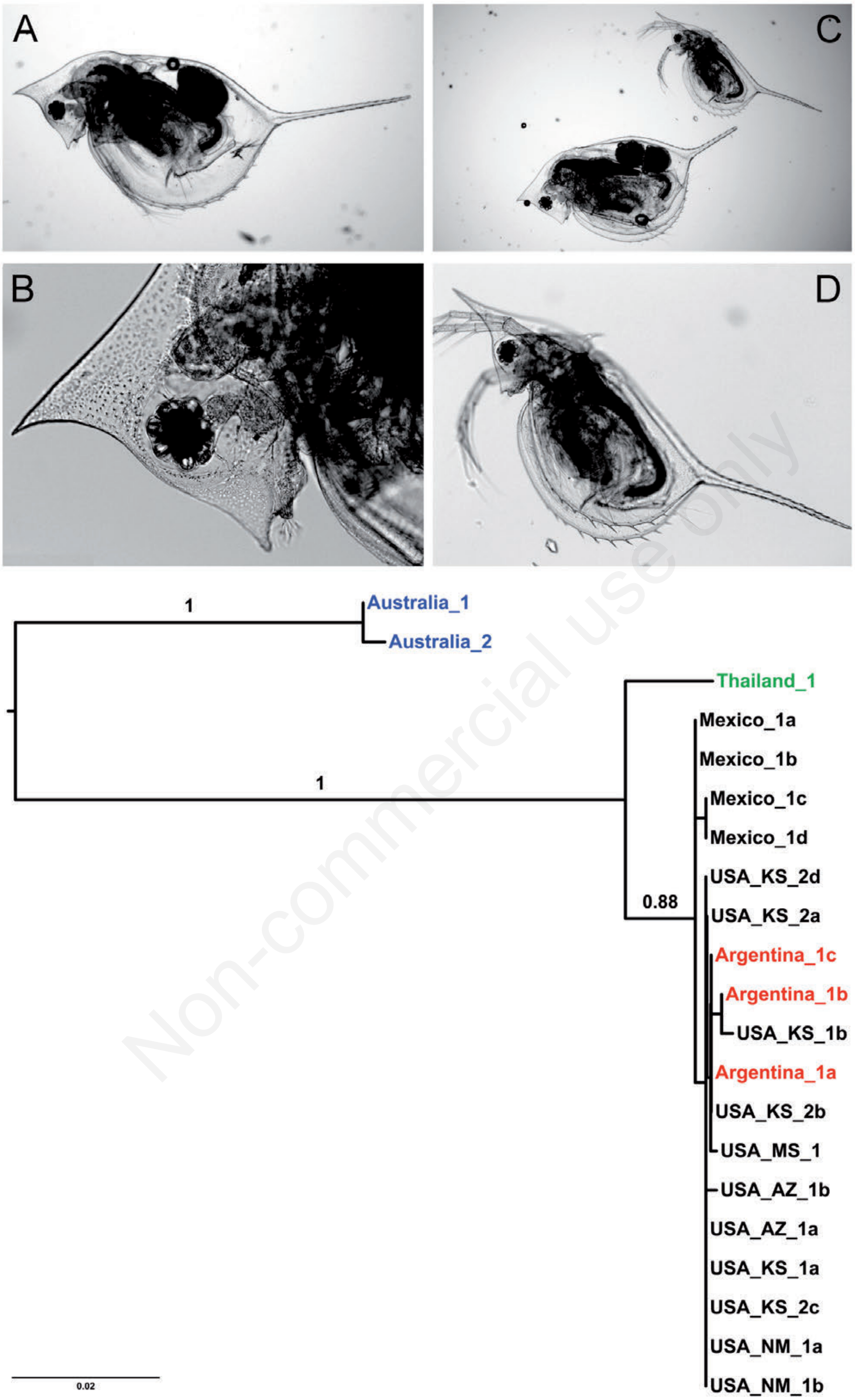

Fig. 2. *Daphnia lumholtzi* in Argentina. A-D) Different adult and juvenile females from a pool adjacent to the Paraná River, between Paraná and Santa Fe, Argentina. E) A Maximum Likelihood phylogram showing the close mitochondrial DNA sequence relationships of Argentinian *D. lumholtzi* to North American specimens. Support values given are nodes are approximate likelihood ratio tests (aLRTs). Blue, Australian specimens; green, Asian specimens; black, North American specimens; red, South American specimens. See Tab. 1 for further details on specimens.

37-38°S in Australia (S coast of New South Wales, see Benzie, 2005) and from the Republic of South Africa at about 30°S (Smirnov, 2008). Also, warm climate fails to predict the absence of this taxon in such countries as Spain, Italy, southern France etc., which are located significantly south from the northernmost border of the *D. lumholtzi* distribution. We failed to detect *D. lumholtzi* beyond the Paraná River system in Argentina or in Chile. As others have noted, temperature, dispersal limitation and other historical and biotic factors can interact in range expansion following invasion.

CONCLUSIONS

Given the rapid expansion and multiple modes of dispersal in North America and now South America, it is possible that there will be further expansion. Perhaps the species will reach about 30°S as it does in the Old World. The South American freshwater ecosystems and fisheries should be monitored closely to assess the possible ecological impact related to this successful invasive cladoceran.

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