



INTERNATIONAL CONFERENCE  
ARAL: PAST, PRESENT & FUTURE  
TWO CENTURIES  
OF THE ARAL SEA INVESTIGATIONS

# Genetic identification of Aral *Artemia* populations

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Aladin<sup>2</sup> and T.J. Abatzopoulos<sup>1</sup>**

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Saint Petersburg, 12-15 October 2009

## INTRODUCTION

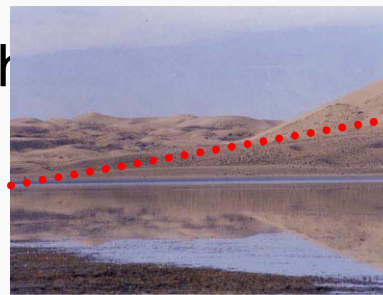
- Crustacea; Branchiopoda; Anostraca
- Cosmopolitan
- Discontinuous distribution – geographical isolation
- Great range of climatic conditions (salinity, temperature, pH, etc)
- Ephemeral environments (astatic, temporal but also permanent)

- Several adaptations have evolved

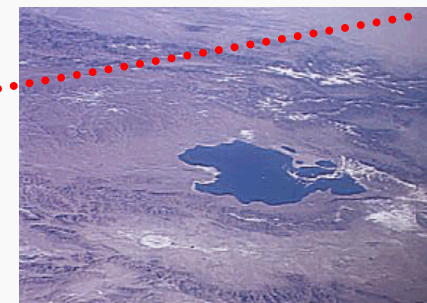


0 m

Saltworks (sea level)



Atacama desert



4500 m

Tibetan High Plateau

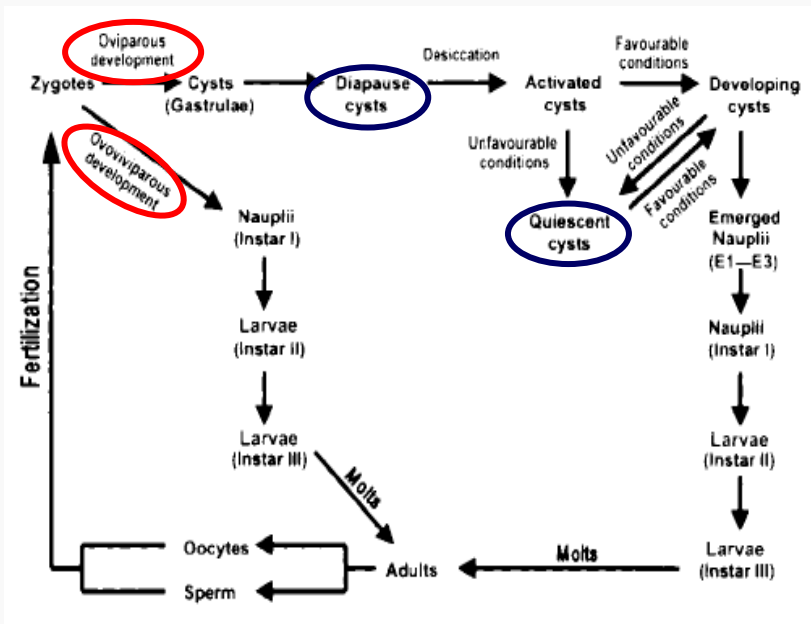
# INTRODUCTION

Developmental Biology 207, 445–456 (1999)  
 Article ID dbio.1998.9138, available online at <http://www.idealibrary.com> on IDEAL®

## The Synthesis of a Small Heat Shock/ $\alpha$ -Crystallin Protein in *Artemia* and Its Relationship to Stress Tolerance during Development

P. Liang<sup>1</sup> and T. H. MacRae

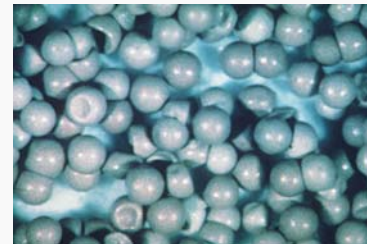
*Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada*



Oviparity → diapause cysts

Ovoviviparity → nauplii

Different level of metabolic arrest:  
 diapause, quiescence



Cysts



Nauplii

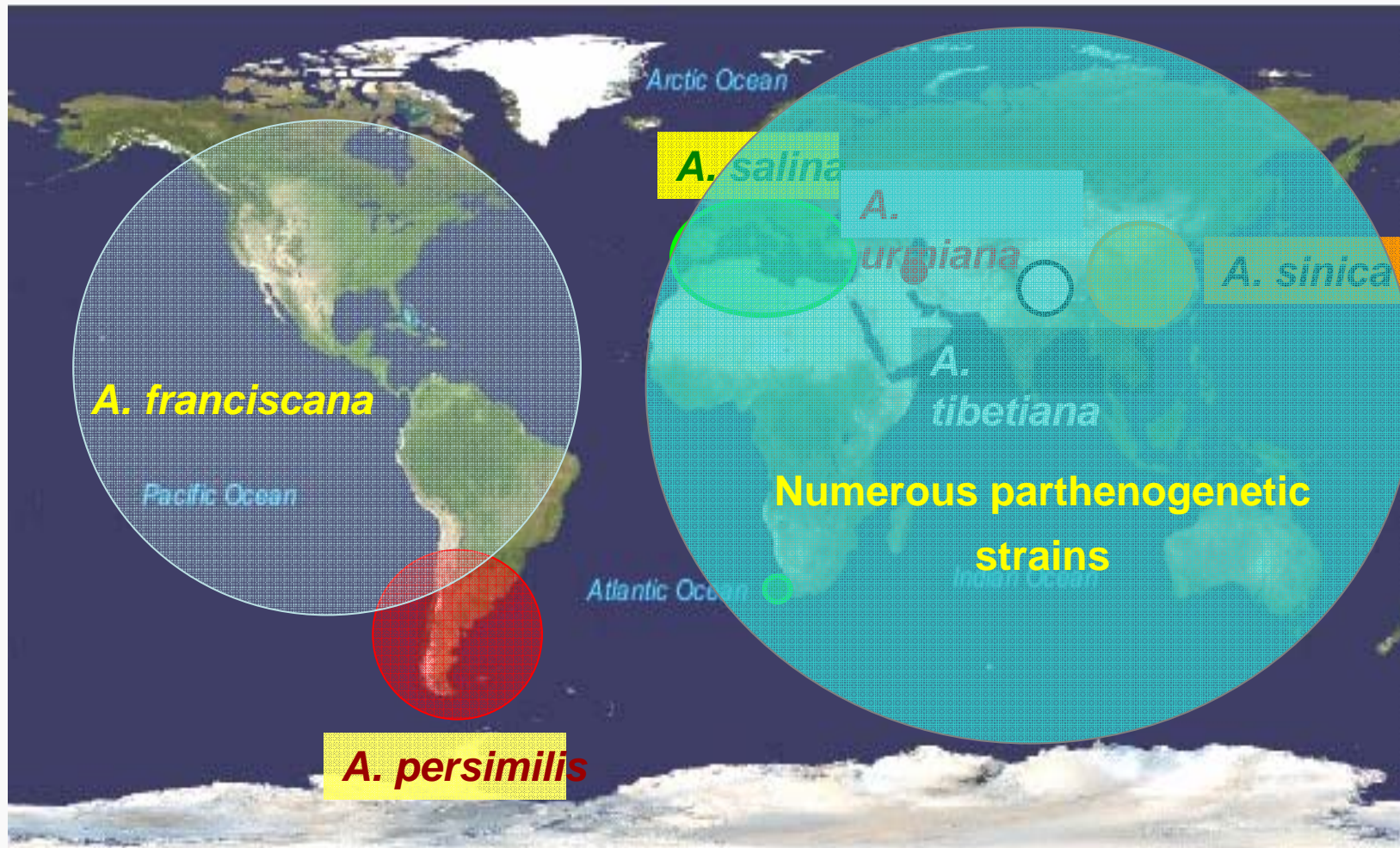
## INTRODUCTION

- 6 bisexual species
  - *A. franciscana*
  - *A. persimilis*
  - *A. salina*
  - *A. urmiana*
  - *A. tibetiana*
  - *A. sinica*
- numerous parthenogenetic strains (Old World)
  - automixis, apomixis (supression of meiosis)
  - existence of clones
  - different ploidy levels
  - avoid the use of the binomen “*A. parthenogenetica*”



# INTRODUCTION

## Species and their distribution



# INTRODUCTION

## The case of *A. urmiana*

Internat. Rev. Hydrobiol.	94	2009	5	540-579
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DOI: 10.1002/irh.200911147

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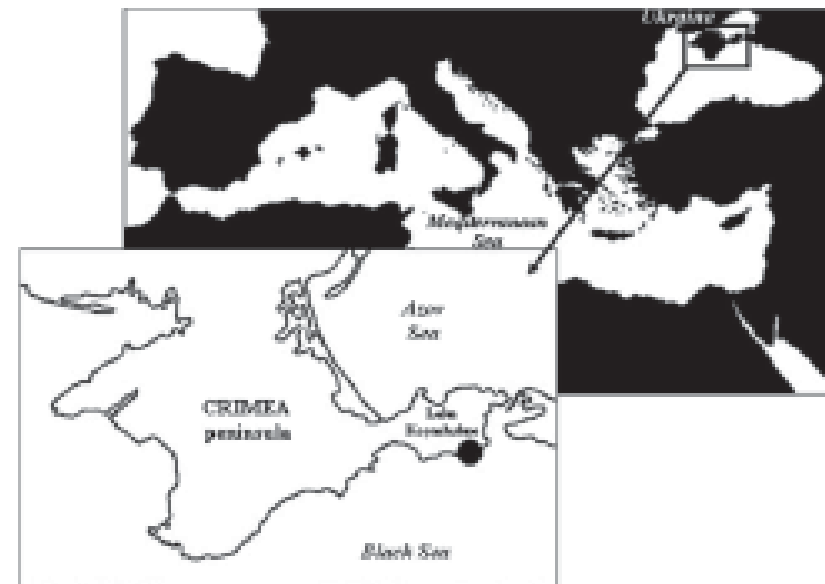
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Research Paper

Updating Geographic Distribution of *Artemia urmiana* GÜNTHER,  
1890 (Branchiopoda: Anostraca) in Europe: An Integrated  
and Interdisciplinary Approach

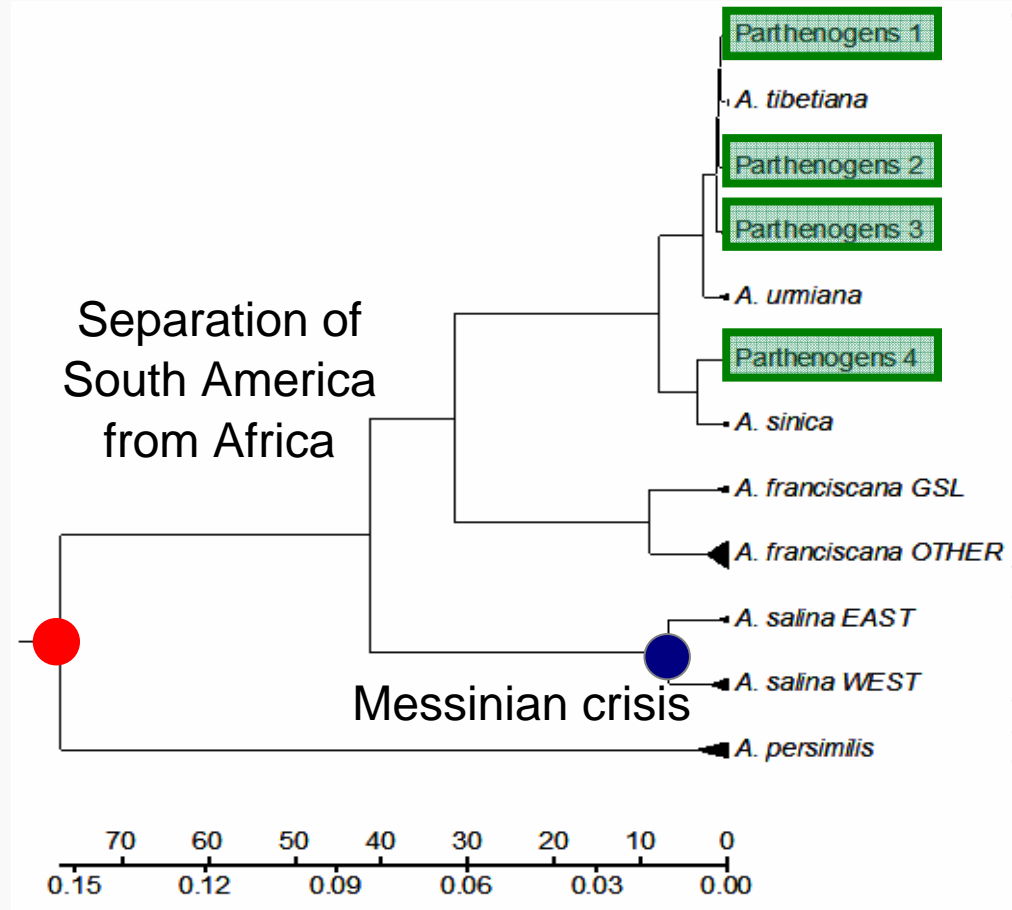


- Multidisciplinary approach
- Recent colonization
- Dispersal by birds

# INTRODUCTION

## Phylogeny

- Global sampling
- Linearized tree
- ITS1 nDNA
- Major geological landmarks
- Multiple origin of parthenogenesis



Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

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EVOLUTION

[www.elsevier.com/locate/ympev](http://www.elsevier.com/locate/ympev)

Molecular phylogenetics and asexuality in the brine shrimp *Artemia*

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Available online 28 April 2006

# INTRODUCTION

## BIODIVERSITY OF THE ARAL SEA AND ITS IMPORTANCE TO THE POSSIBLE WAYS OF REHABILITATING AND CONSERVING ITS REMNANT WATER BODIES

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*Artemia parthenogenetica* (Branchiopoda: Anostraca) from the Large Aral Sea: Abundance, distribution, population structure and cyst production

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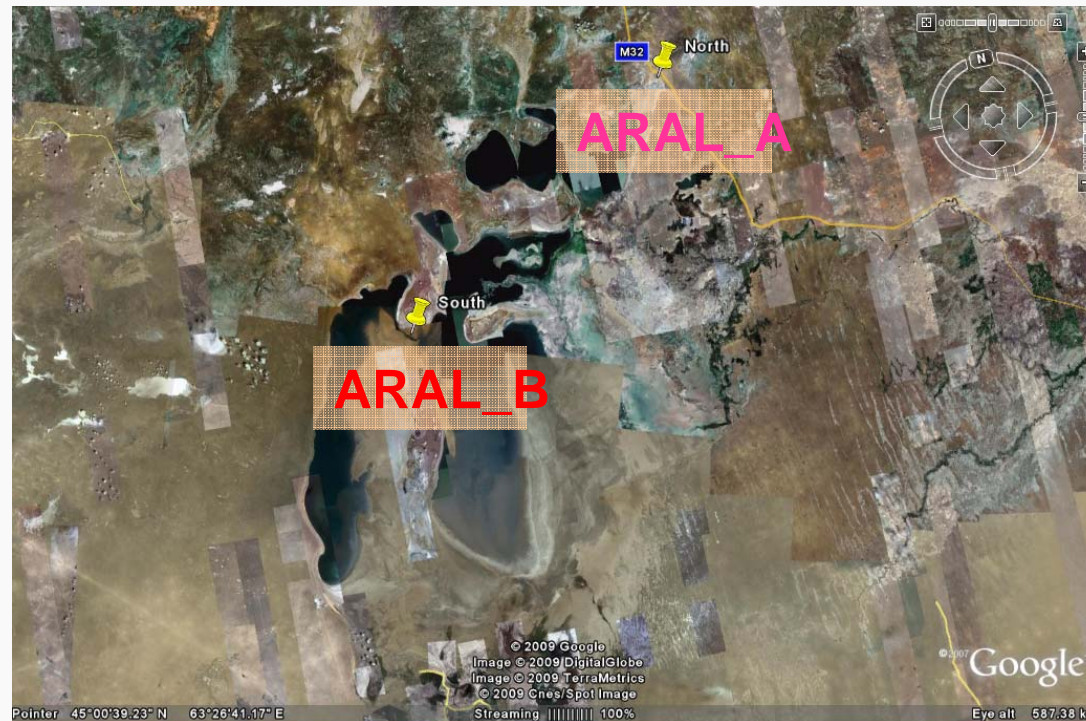
- *Artemia* populations appeared in Large Aral Sea at the end of 20<sup>th</sup> century

- No records of *Artemia* in Small Aral Sea
- *Artemia* occurrence coincides with the increase of salinity in Large Aral Sea
- *Artemia* represents 99% of the total biomass of the zooplankton community



# MATERIALS & METHODS

- Samples analysed
  - Two sites in Aral Sea (Sample A, Sample B)
  - Individuals in ethanol
  - DNA extraction from 10 individuals per sample



## *MATERIALS & METHODS*

- 16S rRNA analysis
  - 2 individuals per sample (**ARAL\_A3, ARAL\_A4, ARAL\_B1, ARAL\_B3**)
  - Universal primers **L2510** and **H3080** (Palumbi *et al.* 1996)
  - PCR and sequencing adopted by Baxevanis *et al.* (2006)
  - 16S rRNA data set comprised of 44 sequences:
    - 39 reference sequences  
(all bisexual species and 6 parthenogenetic populations)
    - 4 sequences obtained from Aral Sea  
(ARAL\_A and ARAL\_B samples)
    - 1 sequence as outgroup  
(*Streptocephalus dorotheae*, Remigio & Herbert, 2000)
  - Alignment, sequence analysis, substitution model selection and phylogenetic reconstruction (ML and MP) followed Baxevanis *et al.* (2006) and Kappas *et al.* (2009)

## *MATERIALS & METHODS*

- Microsatellite analysis
  - 5 individuals per Aral sample genotyped
  - 5 microsatellite loci: **Apdq01**, **02**, **03**, **04** and **05TAIL**
  - PCR conditions according to Muñoz *et al.* (2009)
  - Samples were genotyped in semi-automated sequencer (LiCOR 4100)
  - Microsatellite data set comprised of 76 parthenogenetic individuals
    - Greece (MEM, POL)
    - Israel (EIL)
    - Namibia (NAM)
    - Madagascar (MAD)
    - China (JIA)
    - **ARAL\_A** and **ARAL\_B**
  - Three clonal diversity indices
  - Pairwise genetic distances in PHYLIP (Cavalli-Sforza's) and GENODIVE (Bruvo *et al.*)

## *RESULTS & DISCUSSION*

- 16S rRNA pairwise sequence divergence between **ARAL\_A** and **ARAL\_B** samples was 0.1% (TrN+G model of nucleotide substitution)
- The average sequence divergence between the Aral samples and the rest of parthenogenetic populations was 4%
  - Sequence divergence within parthenogenetic populations: ~7%
  - Sequence divergence within bisexual species: 0.5 – 3.6%
- 16S rRNA phylogeny reconstruction (MP and ML)
- Confirmation of parthenogenetic status of *Aral Artemia* populations
- Not enough resolution within the parthenogens

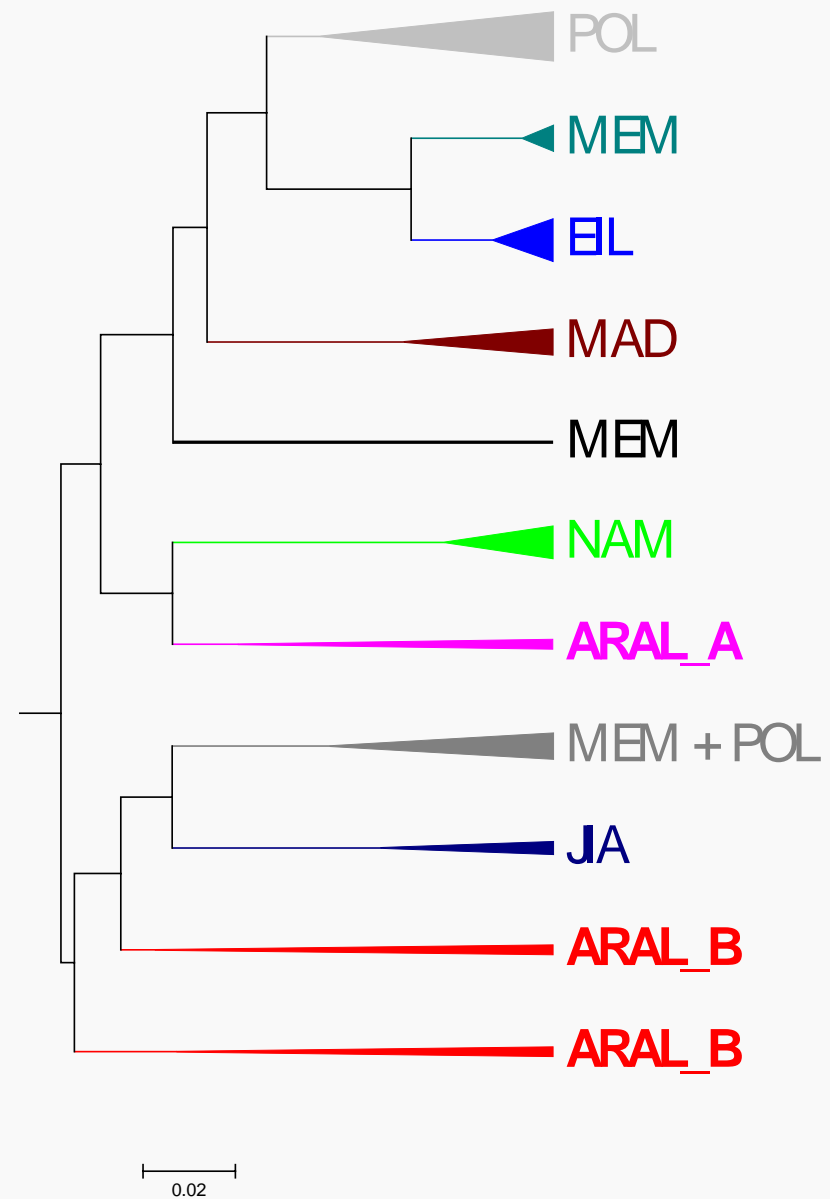
## RESULTS & DISCUSSION

- All loci used are polymorphic
- Aral *Artemia* samples are highly polymorphic compared to the studied parthenogenetic populations

Abbreviation	Population	Latitude	Longitude	Simpson's diversity index	Evenness	Shannon index
MEM	M. Embolon, Greece	40°30'N	22°48'E	0.530	0.649	0.423
POL	Polychnitos, Greece	39°04'N	26°11'E	0.395	0.800	0.244
EIL	Eilat, Israel	29°33'N	34°57'E	0.000	1.000	0.000
NAM	Swakopmund, Namibia	22°40'S	14°34'E	0.000	1.000	0.000
MAD	Ankiembe, Madagascar	12°19'S	49°17'E	0.000	1.000	0.000
JIA	Jiangsu, P. R. China	34°36'N	113°36'E	0.667	1.000	0.321
ARAL_A	Small Aral Sea	46°47'N,	61°40'E	0.833	0.889	0.766
ARAL_B	Large Aral Sea	45°40'N	59°18'E	0.900	0.893	0.991

## RESULTS & DISCUSSION

- Microsatellite distance measurements showed the populations from the Northern part of Aral Sea (**ARAL\_A**) are in different group than those from the Strait (**ARAL\_B**)
- Passive dispersal of cysts: migratory waterfowl or wind



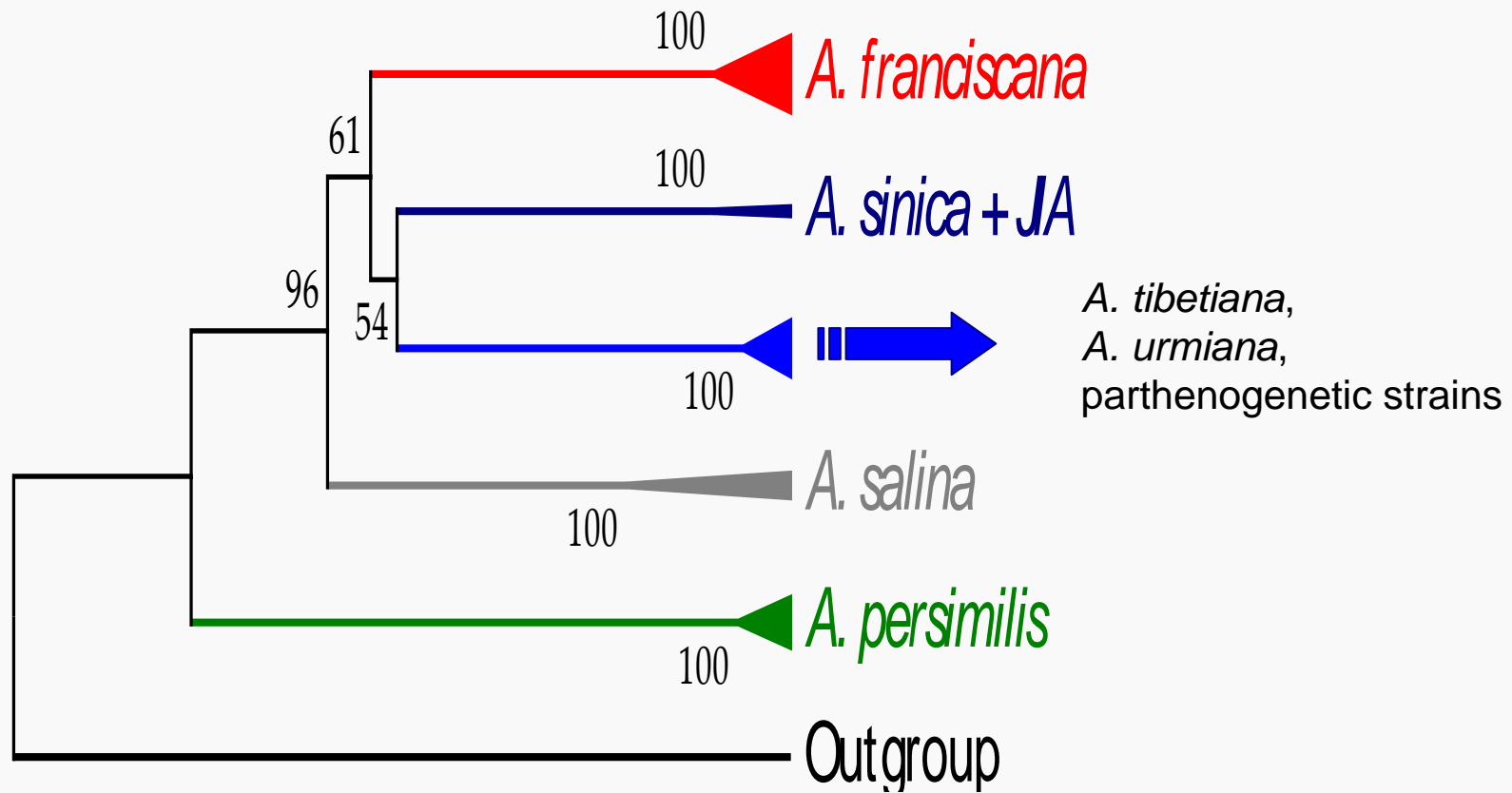
## *RESULTS & DISCUSSION*

- Microsatellites have been proven more powerful than 16S rRNA for resolving phylogenetic relationships of the parthenogenetic *Artemia* populations used
- Preliminary results provide strong evidence for distinct genetic make-up of the two Aral Sea samples
- Aral *Artemia* is parthenogenetic (based on the analysis of the two samples). However, bisexual *Artemia* in Aral Sea cannot be excluded
- More samples and further analyses are needed, therefore, we seek for collaborations

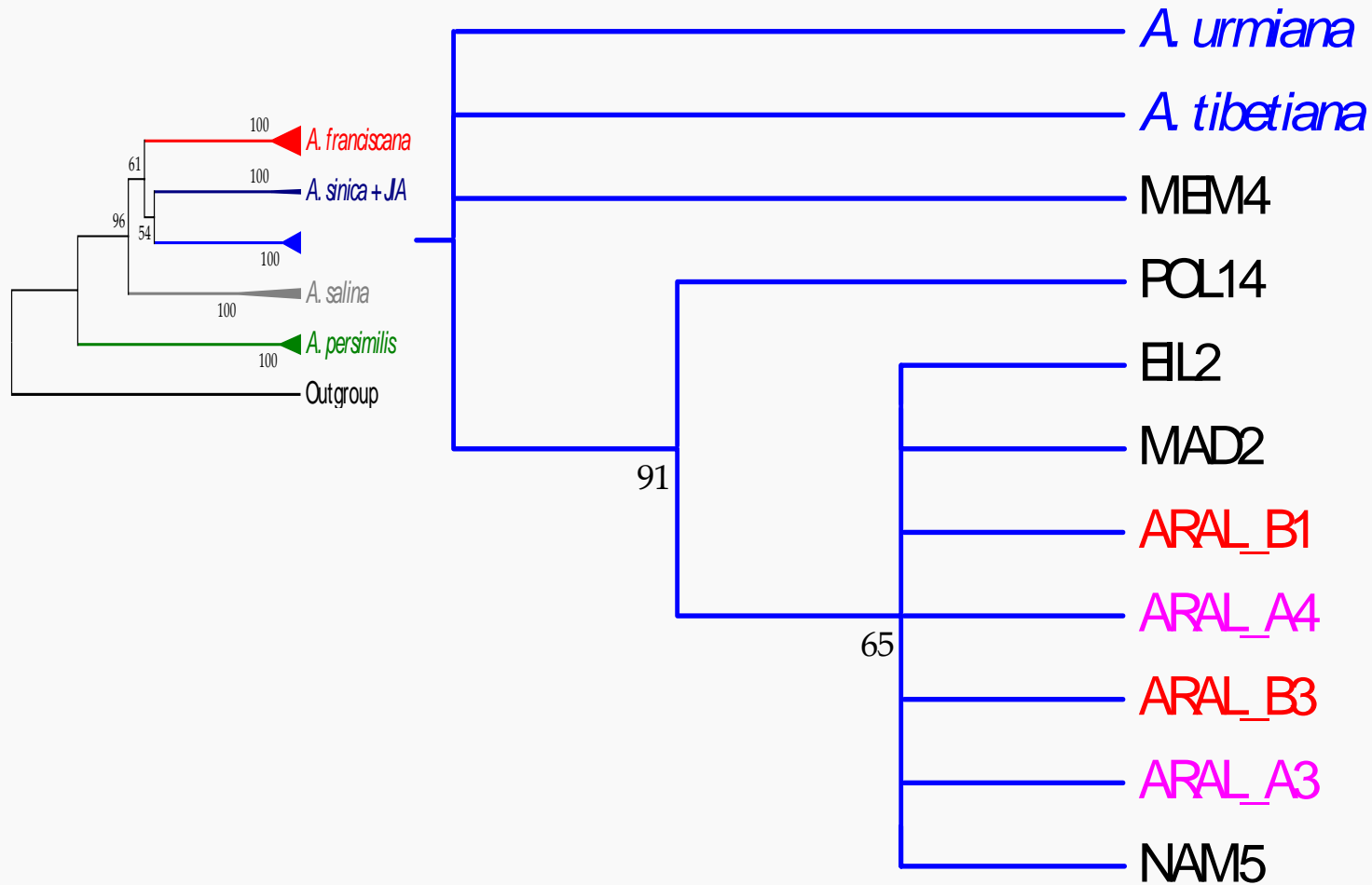
Thank you...



## RESULTS & DISCUSSION



# RESULTS & DISCUSSION



# INTRODUCTION

