Halorubrum halodurans sp. nov., an extremely halophilic archaeon isolated from a hypersaline lake

Paulina Corral,1 Rafael R. de la Haba,1 Cristina Sánchez-Porro,1 Mohammad Ali Amoozegar,2 R. Thane Papke3 and Antonio Ventosa1

1Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain
2Department of Microbiology, Faculty of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran
3Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA

Two extremely halophilic archaea, strains Cb34T and C170, belonging to the genus Halorubrum, were isolated from the brine of the hypersaline lake Aran-Bidgol in Iran. Cells of the two strains were motile, pleomorphic rods, stained Gram-variable and produced red-pigmented colonies. Strains Cb34T and C170 required 25 % (w/v) salts, pH 7.0 and 37 °C for optimal growth under aerobic conditions; 0.3 M Mg2+ was required. Cells of both isolates were lysed in distilled water and hypotonic treatment with <10 % NaCl provoked cell lysis. Phylogenetic analysis based on 16S rRNA gene sequence similarities showed that these two strains were closely related to Halorubrum cibi B31T (98.8 %) and other members of the genus Halorubrum. In addition, studies based on the rpoB gene revealed that strains Cb34T and C170 are placed among the species of Halorubrum and are closely related to Halorubrum cibi B31T, with rpoB gene sequence similarity less than or equal to 95.7 %. The polar lipid patterns of both strains consisted of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl glucosyl diether. The DNA G+C content was 62.1–62.4 mol%. DNA–DNA hybridization studies confirmed that strains Cb34T and C170 constitute a distinct species. Data obtained in this study show that the two strains represent a novel species, for which the name Halorubrum halodurans sp. nov. is proposed. The type strain is Cb34T (=CECT 8745T=IBRC-M 10233T).

The haloarchaea are a group of extremely halophilic, aerobic archaea included in the class Halobacteria (Grant et al., 2001) that comprises 48 genera including the genus Halorubrum, established by McGenity & Grant (1995). Currently, it is the largest haloarchael genus, with 31 validly published species names (Parte, 2015). Species of the genus Halorubrum have been isolated from diverse natural and artificial hypersaline environments such as salterns, salt lakes, coastal sabkhas, soda lakes, saline soils and salt-fermented and salt-preserved food. It is often reported as the dominant genus present in many hypersaline environments; their ubiquity and adaptability to nearly all possible hypersaline conditions has been demonstrated by cultivation and culture-independent methodologies (Ghai et al., 2011; Makhdoumi-Kakhki et al., 2012a; Fernández et al., 2014a, b). Species of this genus are aerobic, chemo-organotrophic and require high concentrations of NaCl in media (e.g. 1.0–5.2 M) for growth. The genus is phenotypically highly variable: its members use many carbon substrates including a wide range of sugars as sources of energy. However, some species use only single carbon sources for growth. They produce bacterioruberin carotenoid pigments that colour colonies pink to red. Most described species are neutrophilic, but some are alkaliphilic and grow optimally at pH 9.5. The major polar lipids are C20C20 and sometimes C20C25.
The pH of the lake brine is neutral, the predominant salts the central desert area of Iran, formed in the Pliocene period. Aran-Bidgol hypersaline lake is a natural ecosystem located in

et al. 2004; Enache advantages of the 16S rRNA gene marker (Walsh reported to be very successful in overcoming the above dis-

strains into species and species into genera, as well as haloarchaea. The MLSA approach is typically a reliable activity the phylogeny of newly discovered strains of Halobacteriaceae is being used to construct phylogenies within the family

rpoB genes investigated previously for evolutionary and taxo-

issues concerning establishing a phylogeny and therefore taxonomy make the 16S rRNA gene unsuitable for these purposes (Ram-Mohan et al., 2014). To overcome some of these limitations, analysis of several housekeeping genes using a multilocus sequencing analysis (MLSA) approach is being used to construct phylogenies within the family Halobacteriaceae, since it resolves and defines with sensitivty the phylogeny of newly discovered strains of haloarchaea. The MLSA approach is typically a reliable method that differentiates individual strains, and groups strains into species and species into genera, as well as family-like relationships within the Halobacteriales (Papke et al., 2011). Among the single copy protein-encoding genes investigated previously for evolutionary and taxonomic studies in haloarchaea, the rpoB gene has been reported to be very successful in overcoming the above disadvantages of the 16S rRNA gene marker (Walsh et al., 2004; Enache et al., 2007; Minegishi et al., 2010; Papke et al., 2011; Fullmer et al., 2014; Ram-Mohan et al., 2014).

Aran-Bidgol hypersaline lake is a natural ecosystem located in the central desert area of Iran, formed in the Pliocene period. The pH of the lake brine is neutral, the predominant salts are NaCl, Na$_2$SO$_4$, MgCl$_2$ and MgSO$_4$ with trace amounts of carbonates, and it is considered a thalassohaline lake (Makhdoumi-Kakhki et al., 2012a). In previous studies of the haloarchaeal population in this lake, several novel genera and species of this group of micro-organisms were isolated and characterized (Amoozegar et al., 2012, 2013, 2014a, b, 2015; Makhdoumi-Kakhki et al., 2012b, c).

In the present study, the properties of two extremely halo-

philic archaea, strains Cb34$^+$ and C170, are described on the basis of standard taxonomic methods following the proposed minimal standards recommended by Oren et al. (1997) for describing novel taxa of the order Halobacteriales. Additionally, we have included an rpoB$^+$ gene phylo-

geny to complement and reinforce our results.

Strains Cb34$^+$ and C170 were isolated from brine of lake Aran-Bidgol (34° 18’–34° 45’ N 51° 33’–52° 10’ E). Isolation of haloarchaea was performed by plating 100 µl brine, as well as using serial dilutions from sediment samples up to 10$^{-5}$, directly on Hv-YPC solid medium (Allers et al., 2004). The plates were incubated aerobically at 37 °C in sealed plastic bags for up to 4 weeks. Representative colonies were picked and transferred to the same medium; after successive streaking, two pure cultures designated strains Cb34$^+$ and C170 were obtained. They produced convex, smooth, round and red-pigmented colonies (0.5–1.0 mm diameter) with an entire edge. The strains were grown routinely in M1 20 % medium (Rodrı´ guez-Valera et al., 1980), prepared using a salt mixture designated SW 30 % (w/v) stock solution (Subow, 1931), which consists of (per litre): 234 g NaCl, 39 g MgCl$_2$.6 H$_2$O, 61 g MgSO$_4$. 7H$_2$O, 1 g CaCl$_2$, 6 g KCl, 0.2 g NaHCO$_3$ and 0.7 g NaBr. This solution was supplemented with 0.2 % (w/v) yeast extract, 0.1 % (w/v) Casamino acids and 0.005 % (w/v) sodium pyruvate. The medium was adjusted to pH 7.0–7.2. For solid media, 2.0 % (w/v) agar was used when necessary. The strains were maintained on the same medium in slant tubes; for long-term preservation, the strains were prepared as cryotubes for freezing at (−80 °C in suspensions as follows: to 750 µl fresh culture (OD$_{600}$ 0.8–1.0), 250 µl glycerol/SW 30 (80 : 20, v/v) was added (Dyll-Smith, 2009).

Cell morphology and motility were examined in liquid medium after 7 days of growth by optical and phase-

contrast microscopy (BX41; Olympus). Gram staining was performed using acetic acid-fixed samples, as described by Dussault (1955). Cells of strains Cb34$^+$ and C170 were observed as rod-shaped and flattened pleomorphic cells (Fig. S1, available in the online Supplementary Material); they were motile and stained Gram-variable.

The growth and optimum requirements for NaCl, Mg$^{2+}$, pH and temperature were determined in the routine medium M1, changing the recipe for testing growth at different concentrations of salts. The range of NaCl (5–30 %, w/v) was tested at intervals of 5 %. Strains Cb34$^+$ and C170 grew in the presence of 20–30 % (w/v) total salts (optimum 25 %, w/v). Cells of both isolates were lysed in distilled water as well as after hypotonic treatment with less than 10 % (w/v) NaCl. The magnesium range was tested using MgCl$_2$ (0–10 %, w/v) at intervals of 1 % (w/v). Routine cultivation was performed at 37 °C and pH 7.2. Strains Cb34$^+$ and C170 have a minimal magnesium ion requirement of 0.3 M Mg$^{2+}$. The pH range for growth was assayed at pH 5.5–10.0 at intervals of 0.5
Halorubrum cibi references for comparative purposes for subsequent testing: medium M1 prepared at 25 % (w/v) total salts, pH 7.0. All phenotypic tests were carried out using the routine aerobic conditions.

L-arginine by adding 3 % KNO₃ or 4 % L-arginine, 8 from 20 to 45 °C, and the optimum was 37 °C under aerobic conditions.

All phenotypic tests were carried out using the routine medium M1 prepared at 25 % (w/v) total salts, pH 7.0 and 37 °C. The following type strains were used as references for comparative purposes for subsequent testing: Halorubrum cibi JCM 15757ᵀ, Hrr. aquaticum EN-2ᵀ, Hrr. kocurii CECT 7322ᵀ, Hrr. alkaliphilum JCM 12358ᵀ, Hrr. tibetense JCM 11889ᵀ, Hrr. lipoleticum JC 13559ᵀ and Hrr. saccharovorum JCM 8865ᵀ.

Anaerobic growth was tested in the presence of nitrate and L-arginine by adding 3 % KNO₃ or 4 % L-arginine, respectively, to the medium in filled stoppered tubes, as well as plates of cultures incubated for 10 days at 37 °C in an anaerobic jar (Oren et al., 1997). Strains Chb34ᵀ and C170 were unable to use nitrate (with or without L-arginine) or DMSO as alternative electron acceptors under anaerobic conditions. Catalase activity was determined by adding a 1 % (w/v) H₂O₂ solution to colonies on solid medium. The oxidase test was performed using a DrySlide assay (Difco). Both strains were oxidase- and catalase-negative. Hydrolysis of starch, gelatin, aesculin, casein and Tween 80 was assessed as described by Barrow & Feltham (2003). Test for indole production from tryptophan and urea were performed as described by Gerhardt et al. (1994). The methyl red, Voges–Proskauer and Simmons’ citrate tests were performed as described by Oren et al. (1997). H₂S formation was determined by monitoring the production of a black sulfide precipitate in solid M1 medium containing 0.5 % (w/v) sodium thiosulfate. Reduction of nitrate was detected by using sulfanilic acid as the only source of carbon, nitrogen and energy, a medium containing 0.05 % (w/v) yeast extract and supplemented with 1 % (w/v) of the tested substrate (sterilized separately) was assessed as described by Ventosa et al. (1999). The production of acid from different carbohydrates was tested in a medium with 0.5 % (w/v) yeast extract and supplemented with 1 % (w/v) of the tested carbohydrate (Oren et al., 1997). Hydrolysis of different compounds and utilization of substrates is detailed in the species description. Strains Chb34ᵀ and C170 shared similar growth conditions and metabolic requirements; there were no significant phenotypic differences between them. Additionally, there were no discrepancies in phenotypic tests determined in our laboratory for the reference strains and those reported previously for closely related species.

Differential features of strains Chb34ᵀ and C170 from the type strains of closely related species are shown in Table 1.

Antimicrobial compound sensitivity tests were performed by spreading a culture suspension on plates of solid medium M1 20 % and applying discs (BD) soaked with antimicrobial compounds following the Bauer–Kirby technique (Bauer et al., 1966) as described by Ventosa et al. (1999). Discs containing anisomycin (50 μg) were prepared in our laboratory and applied in the same way. Strains Chb34ᵀ and C170 were sensitive to anisomycin (50 μg), bacitracin (10 IU), novobiocin (30 μg), rifampicin (5 μg), erythromycin (15 μg), streptomycin (10 μg) and trimethoprim/sulfamethoxazole (1.25/23.75 μg) and resistant to ampicillin (10 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), neomycin (30 IU), gentamicin (10 IU), kanamycin (30 μg), penicillin G (10 IU) and tetracycline (30 μg).

Genomic DNA of strains Chb34ᵀ and C170 was obtained by the method of Marmur (1961). The 16S rRNA gene was amplified by PCR (Sambrook & Russell, 2001) using universal primers as described previously (DeLong, 1992; Arahal et al., 1996). The complete rpoB (RNA polymerase subunit B’) gene sequence of strain Chb34ᵀ was obtained from the genome sequence (Fullmer et al., 2014). The rpoB gene of strain C170 was amplified by PCR, using the primers designed by Fullmer et al. (2014) and Ram Mohan et al. (2014). The sequencing primer M13 (18 bp) was added to the 5’ end of the degenerate primers to sequence PCR products more efficiently (Fullmer et al., 2014; Ram-Mohan et al., 2014). The PCR products were separated by gel electrophoresis with agarose (1 % w/v) and gels were stained with ethidium bromide. An exACTGene mid-range plus DNA ladder (Fisher Scientific International Inc.) was used to estimate the size of the amplicons.

Amplicons were purified using the Wizard SV Gel and PCR Cleanup system (Promega). The purified 16S rRNA gene PCR product was sequenced by StabVida (Oeiras, Portugal) and the product of the rpoB gene by Genewiz Inc. (Boston, USA) using Sanger sequencing technology. Sequencing reactions were carried out in both directions by the dideoxynucleotide chain-termination method using the BigDye terminator kit version 3.1 from Applied Biosystems. Sequencing products were purified by gel filtration and resolved in an ABI 3130XL or ABI 3730XL DNA Analyzer sequencer, according to the manufacturer’s instructions. The nucleotide sequences of the 16S rRNA genes of strains Chb34ᵀ and C170 were aligned with Chro-masPro software version 1.7.6 and ARB 5.5 software package (Ludwig et al., 2004). Sequence similarity was analysed by comparing the 16S rRNA gene sequences of strains Chb34ᵀ and C170 with known sequences from the EzTaxon-e database (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012). The similarity analysis based on complete 16S rRNA gene sequences showed that the sequences of strains Chb34ᵀ (1402 bp) and C170 (1379 bp) were
99.8 % identical and were closely related to those of *Hrr. cibi* B31T (98.8 % similarity), *Hrr. aquaticum* EN-2T (98.5 %), *Hrr. kocurii* BG-1T (97.4 %), *Hrr. alkaliphilum* DZ-1T (97.3 %), *Hrr. tibetense* 8W8T (97.3 %) and *Hrr. lipolyticum* 9-3T (97.1 %). Lower similarities were obtained with *Hrr. tibetense* JCM 11889T; *Hrr. alkaliphilum* JCM 13559T. Data are from this study unless indicated.

Phylogenetic study based on 16S rRNA gene sequence comparison was performed by reconstructing phylogenetic trees using the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms (Saitou & Nei, 1987) with the *ARB* program package version 5.5 (Ludwig et al., 2004). Maximum-likelihood analysis was performed with RAxML 7.0.4 using the general time-reversible (GTR) model of nucleotide substitution (Stamatakis et al., 2005) with Gamma and invariable site estimations. Base-frequency filters were applied in the sequence-comparison analysis, and the effects on the results were evaluated. To evaluate the robustness of the phylogenetic tree, a bootstrap analysis (1000 replications) was performed (Felsenstein, 1985). The phylogenetic tree based on the 16S rRNA gene reconstructed by the maximum-likelihood method confirmed that strains Cb34T and C170 are related to *Hrr. cibi* B31T and that they fall within the cluster constituted by species of the genus *Halorubrum*. Topologies of phylogenetic trees inferred by using the neighbour-joining and maximum-parsimony algorithms were highly similar to that of the tree reconstructed by the maximum-likelihood method (Fig. 1).

The phylogeny based on the *rpoB* gene was assessed after recovering gene sequences from the GenBank database. In some cases, sequences were obtained from the genomes of the type strains recovered from the NCBI ftp repository. The top-scoring *BLASTN* hits. Multiple-sequence alignments were created after taking into account the amino acid alignments for the *rpoB* protein-encoding gene generated by translating this gene to protein sequences in...
SeaView (Gouy et al., 2010), aligning the proteins using MUSCLE (version 3.8.31) (Edgar, 2004) with its refine function and then reverting back to the nucleotide sequences. Alignments were edited using Mesquite version 2.75 (Maddison & Maddison, 2011). The best model of evolution was determined by calculating the Akaike information criterion with correction for small sample size (AICc). The jModelTest 2.1.4 (Darriba et al., 2012) program was used to compute likelihoods from the nucleotide alignment and to perform the AICc test (Akaike, 1974). The AICc reported the best-fitting model to be TVM + Gamma estimation + invariable site estimation. A maximum-likelihood phylogeny was generated from the multiple-sequence alignment using the PhyML version 3.0_360-500 (Guindon et al., 2010) with 1000 bootstrap replicates. The model and parameters used in PhyML corresponded to the one favoured by the jModel test. The number of nucleotide differences in pairwise comparisons was determined using MEGA 5 (Tamura et al., 2011).

The rpoB phylogenetic tree showed that strains Cb34 and C170 clustered with the species of Halorubrum but formed an independent and defined branch, clearly separated from the other species that constitute this genus (Fig. 2).

The G+C content of the genomic DNA was determined by thermal denaturation using the melting midpoint ($T_m$) (Marmur & Doty, 1962) using the equation of
Owen & Pitcher (1985). The DNA G+C content of strains Cb34\textsuperscript{T} and C170 was 62.1–62.4 mol%, within the range reported for the genus *Halorubrum*, 60.2–71.2 mol% (McGenity & Grant 2001; Roh & Bae 2009).

DNA–DNA hybridization (DDH) studies between strains Cb34\textsuperscript{T} and C170 and type strains of the phylogenetically most closely related species of the genus *Halorubrum* were performed in triplicate by the competition procedure of the membrane filter method of Johnson (1994). DDH were performed in triplicate by the competition procedure of membrane polar lipids of halophilic archaea described by Corcelli & Lobasso (2006); the extracts were dried carefully using a SpeedVac Thermo Savan SPD111V before charred by heating at 160 °C (Kates, 1986). The glycolipids the conclusion that the two strains are genotypically distinct and should be assigned to a different species.

Cell biomass of strains Cb34\textsuperscript{T} and C170 for chemotaxonomic analyses was obtained after 10 days of aerobic incubation in M1 liquid medium under optimal conditions: 25 % (w/v) NaCl, 37 °C and pH 7.0. The strains used for comparisons were cultured according to the authors' description for each strain and standardizing to the same incubation conditions. Polar lipids were extracted with chloroform/methanol following the method for extraction of membrane polar lipids of halophilic archaea described by Corcelli & Lobasso (2006); the extracts were dried carefully using a SpeedVac Thermo Savan SPD111V before weighing and then dissolved in chloroform to obtain a concentration of 10 mg lipid ml\textsuperscript{−1}. Total lipid extracts were analysed by one-dimensional HPTLC on Merck silica-gel plates crystal back (Merck art. 5626; 10 x 20 cm); the plates were eluted in the solvent system chloroform/90 % methanol/acetic acid (65 : 4 : 35, by vol.) (Angelini et al., 2012; Corral et al., 2013). To detect all polar lipids, the plate was sprayed with sulfuric acid, 5 % in water, and charred by heating at 160 °C (Kates, 1986). The glycolipids

**Fig. 2.** Maximum-likelihood tree based on rpoB\textsuperscript{\textprime} gene sequences showing the phylogenetic relationship between strains Cb34\textsuperscript{T} and C170 isolated from the lake Aran-Bidgol and other members of the genus *Halorubrum*. Bootstrap values >70 % are indicated. *Halogeometricum borinquense* DSM 11551\textsuperscript{T} and *Halofexx volcanii* NCIMB 2287\textsuperscript{T} were used as an outgroup. Bar, 10 % substitution.
appear as purple spots and the remaining polar lipids as brown spots after prolonged heating; alternatively, the plate was sprayed with a solution of primuline and lipids were detected upon excitation by UV light (336 nm) (Fuchs et al., 2007). Furthermore, the following reagents were used in order to identify the chemical nature of the lipids present in the TLC bands: molybdenum blue Sigma spray reagent for phospholipids (Kates, 1986), azure A/sulfuric acid for sulfatides and sulfoglycolipids (Kean, 1968) and ninhydrin in acetone/lutidine (9 : 1) for free amino groups.

TLC of the polar lipids (Fig. S2) revealed that strains Cb34T and C170 possessed PG, PGP-Me, PGS and a glycolipid that was chromatographically identical to sulfated mannosyl glucosyl diether (S-DGD) as major lipids. Diphosphatidylglycerol was also a minor component. The polar lipid profiles of strains Cb34T and C170 possess all the major lipids described for neutrophilic species of the genus Halorubrum (McGenity & Grant, 1995); however, they lack three minor phospholipid components identified beside PGS and PGP-Me. The polar lipid pattern observed in both strains is similar to the profile of Hrr. cibi JCM 15757T, but the presence of the minor phospholipids not observed in the closely related type strain is in agreement with the genotypic data and provides further evidence to indicate that these strains may represent a different species.

A polyphasic approach including phylogenetic analyses using 16S rRNA and rpoB gene sequence comparisons, polar lipid profiles, DDH and detailed phenotypic characterization confirms that the two strains represent a novel species of the genus Halorubrum, for which the name Halorubrum halodurans sp. nov. is proposed.

Description of Halorubrum halodurans sp. nov.

Halorubrum halodurans (ha.lo.du.‘rans. Gr. n. halos salt; L. part. adj. durans enduring; N.L. part. adj. halodurans salt-enduring, resisting).

Cells are motile, pleomorphic rods, 5.0–10 × 1.0–4.0 μm, and occur singly without grouping. Gram-stain-variable; in young cultures, most cells are Gram-stain-negative, while a few cells are observed as Gram-stain-positive. Gas vesicles are not observed inside cells. Colonies on solid medium after incubation at 37 °C for 10 days are circular, with regular edges, smooth, convex and 0.5–1 mm in diameter. During the first 5 days, colonies appear pink, and the colour gradually intensifies to red on incubation over subsequent days. Cells are lysed in distilled water; hypotonic treatment with less than 10 % (w/v) NaCl induces cell lysis. Has a minimal magnesium ion requirement of 0.3 M Mg2+. The species is neutrophilic, growing at pH 6.0–8.0 with an optimum at pH 7.0–7.2. Requirements for growth on NaCl are 20–30 % (w/v) with an optimum of 25 % (w/v). Grows at 20–45 °C (optimum 37 °C). Chemo-organotrophic and strictly aerobic. Oxidase and catalase activities are detected. Anaerobic growth does not occur with nitrate or L-arginine. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are not produced. H2S is produced from sodium thiosulfate or cysteine; indole is not produced from tryptophan. Nitrate is reduced to nitrite, but nitrite is not reduced further and no gas is formed. Methyl red and Voges–Proskauer tests are negative, and citrate is not utilized. Extracellular hydrolysis of Tween 80, starch, DNA, gelatin and casein is not observed. Tests for urease and phosphatase activities are negative. The following substrates are utilized for growth as sole sources of carbon and energy: d-glucose, sucrose, d-mannose, trehalose, glycerol, d-mannitol, acetic acid, glutamate, lactate, malate, pyruvate, succinate and propionate. d-Galactose, d-fructose, d-ribose, d-xylose, lactose, maltose, sorbose, sorbitol, raffinose, l-arabinose, rhamnose, fumarate and citrate are not used as sole sources of carbon and energy. The following amino acids are used as sole sources of carbon, nitrogen and energy: L-serine, threonine, glycine, asparagine and L-lysine. Isoeleucine, alanine, arginine and ornithine are not used as sole sources of carbon, nitrogen and energy. The polar lipid pattern consists of PG, PGP-Me, PGS and S-DGD as major lipids. Diphosphatidylglycerol and minor phospholipidic components are present at low levels. The DNA G+C content is 62.1–62.4 mol% (Tm).

The type strain is Cb34T (=CECT 8745T=IBRC-M 10233T), isolated from sediment of the hypersaline lake Aran-Bidgol in Iran. The DNA G+C content of the type strain is 62.1 mol% (Tm).

Acknowledgements

This work was supported by grants from the Spanish Ministry of Economy and Competitiveness (CGL2013-46941-P) and from the Andalusian Council (P10-CVI-6226), both with European Funds (FEDER) (to A. V.), the National Science Foundation (award numbers DEB0919290 and DEB0830024) and the NASA Astrobiology: Exobiology and Evolutionary Biology Program (grant no. NNX12AD70G) (to R. T. P.) and the Iranian Biological Resource Centre (IBRC) (MI-1388-04) (to M. A. A.).

References


