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Received: 12 July 2023 | Revised: 29 November 2023 | Accepted: 9 December 2023

DOI: 10.1002/yea.3920

RESEARCH ARTICLE

Fantastic Yeast

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Nakazawaea atacamensis f.a., sp. nov. a novel nonconventional fermentative ascomycetous yeast species from the Atacama Desert

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Funding information
Fondo Nacional de Desarrollo Científico y Tecnológico; INICIATIVA CIENTIFICA MILENIO; Universidad de Santiago de Chile; Conselho Nacional de Desenvolvimento Científico e Tecnológico

Abstract

In this study, we describe *Nakazawaea atacamensis* f. a., sp. nov., a novel species obtained from *Neltuma chilensis* plant samples in Chile's hyperarid Atacama Desert. In total, three strains of *N. atacamensis* were obtained from independent *N. chilensis* samples (synonym *Prosopis chilensis*, Algarrobo). Two strains were obtained from bark samples, while the third strain was obtained from bark-exuded gum from another tree. The novel species was defined using molecular characteristics and subsequently characterized with respect to morphological, physiological, and biochemical properties. A neighbor-joining analysis using the sequences of the D1/D2 domains of the large subunit ribosomal RNA gene revealed that *N. atacamensis* clustered with *Nakazawaea pomicola*. The sequence of *N. atacamensis* differed from closely related species by 1.3%–5.2% in the D1/D2 domains. A phylogenomic analysis based on single-nucleotide polymorphism's data confirms that the novel species belongs to the genus *Nakazawaea*, where *N. atacamensis* clustered with *N. peltata*. Phenotypic comparisons demonstrated that *N. atacamensis* exhibited distinct carbon assimilation patterns compared to its related species. Genome sequencing of the strain ATA-11A-B^T revealed a genome size of approximately 12.4 Mbp, similar to other *Nakazawaea* species, with 5116 protein-coding genes annotated using InterProScan. In addition, *N. atacamensis* exhibited the

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capacity to ferment synthetic wine must, representing a potential new yeast for mono or co-culture wine fermentations. This comprehensive study expands our understanding of the genus *Nakazawaea* and highlights the ecological and industrial potential of *N. atacamensis* in fermentation processes. The holotype of *N. atacamensis* sp. nov. is CBS 18375^{T}. The Mycobank number is MB 849680.

KEYWORDS

Atacama, fermentation, novel species, yeast

1 | INTRODUCTION

Yeasts are known for their ubiquitous presence in various biomes, where they inhabit a wide range of substrates and environments, such as tropical forests (Morais et al., 2006), temperate forests (Mozzachiodi et al., 2022), hyperarid deserts, and cold habitats (Buzzini et al., 2018). Hyperarid desert habitats are characterized by challenging conditions such as low oxygen and water levels, extreme temperatures, and exposure to ultraviolet (UV) radiation (Houston & Hartley, 2003; Schulze-Makuch et al., 2018). To survive in these conditions, yeasts have evolved various adaptation mechanisms. For instance, they produce carotenoid pigments and mycosporines to withstand UV radiation, and possess aquaporins to counteract sudden osmotic changes (Aponte-Santamaría et al., 2017). However, despite the importance of yeast diversity, most studies in hyperarid deserts have focused on bacteria, neglecting the role of yeasts in extreme environments such as deserts (Azua-Bustos et al., 2018; Drees et al., 2006; Finstad et al., 2017).

Exploring microbial diversity in inhospitable habitats represents a promising strategy for discovering novel yeast species with valuable biotechnological potential. In this regard, ethanol-tolerant yeasts demonstrated remarkable abilities that revolutionized biotechnology industries (Cubillos et al., 2019; Libkind et al., 2011). For instance, a recent bioprospecting study led to the identification of new *Saccharomyces* species, including *S. eubayanus*, which has proven valuable for the brewing industry (Eizaguirre et al., 2018; Libkind et al., 2011; Nespolo et al., 2020; Peris et al., 2016). Similarly, the recent advent of non-*Saccharomyces* species from different natural environments is becoming an interesting biological resource to develop novel fermented beverages (Canonico et al., 2019; Villarreal et al., 2022). However, the occurrence and diversity of culturable yeasts in hyperarid and desert areas have been largely overlooked (Wei et al., 2022).

The Atacama Desert, situated in northern Chile (19°–27°S), stands as the driest and oldest desert on Earth, with an average annual precipitation of less than 20 mm, representing an extreme environment for life (Ewing et al., 2006; McKay et al., 2003). Despite these harsh conditions, a few resilient woody plants and perennial grasses manage to thrive in this environment (Carrasco-Puga et al., 2021). Similarly, the Atacama Desert soil harbors diverse microorganisms, particularly in surficial salt crusts (Wierzchos et al., 2011) and in association with plants (Eshel et al., 2021). Previous studies have predominantly focused on bacteria in the field of astrobiology, aiming to gain fundamental insights into terrestrial desert environments and their microbiota (Azua-Bustos et al., 2012; Pulschen et al., 2015). However, investigations on yeast communities associated with the flora of the Atacama Desert, thriving under extremely arid conditions, remain scarce.

Take-away

- A novel species, *Nakazawaea atacamensis* sp. nov., was isolated from *Neltuma chilensis* (Algarrobo) samples in the Atacama Desert of Chile.
- The sequence of *N. atacamensis* differs from closely related species by 1.3%–5.2% in the D1/D2 domains.
- *N. atacamensis* exhibited the capacity to ferment synthetic wine must, suggesting its potential for wine fermentations.

Historically, the indigenous people of the Atacama region produced spontaneously fermented beverages, which typically exhibit low ethanol levels. These beverages are created through the natural fermentation of leguminous tree pods and fruit juices, providing a source of calories, proteins, vitamins, minerals, and diverse bioactive compounds (Sciammaro & Ribotta, 2016). For instance, "Aloja" is an alcoholic beverage made by fermenting mashed pods of the *Neltuma* (synonym *Prosopis*) tree, locally known as Algarrobo (Pardo & Pizarro, 2015; Sciammaro et al., 2022). This practice suggests the presence of indigenous ethanol-tolerant yeasts within naturally fermented musts, indicating a promising opportunity for the discovery of novel fermentative yeasts within the local vegetation of the Atacama Desert.

In this study, our aim was to isolate ethanol-tolerant yeasts from the Atacama Desert's flora using an enrichment culture approach. We successfully obtained three yeast strains from various tree samples, one from bark-exuded gum and two from different *Neltuma chilensis* tree barks. Phylogenetic analyses support classifying these strains as a previously unknown yeast species within the *Nakazawaea* genus. We propose the name *Nakazawaea atacamensis* sp. nov. for this novel

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species. Additionally, we demonstrate its potential in wine fermentations and analyze metabolite production and sugar consumption under wine fermentation conditions.

2 | MATERIALS AND METHODS

2.1 | Sampling and yeast isolation

The sampling for this study took place in the Atacama Desert near the San Pedro de Atacama village (22° 55′ S, 68° 12′ W) in January 2021. The sampling strategy involved identifying tree species and collecting various types of samples from each tree. Briefly, 5 g of bark, bark-exuded gum, pods, and flowers of Algarrobo (*N. chilensis* [Molina] C. E. Hughes & G. P. Lewis), Tamarugo (*Strombocarpa tamarugo* [Phil] C. E. Hughes & G. P. Lewis), and Chañar (*Geoffroea decorticans* [Gill., ex Hook & Arn.] Burkart) were collected. A total of 22 samples were collected in aseptic conditions and transferred to tubes containing 10 mL of selective enrichment medium composed of 0.67% yeast nitrogen base (YNB; Difco) supplemented with 2% (wt/vol) glucose and 4% (vol/vol) ethanol. This medium was specifically designed to exclusively select for ethanol-tolerant yeast species (Villarreal et al., 2022). The tubes were incubated at 25°C without agitation for a period of 14 days. After incubation, 100 µL aliquots were spread onto yeast-extract peptone dextrose (YPD) agar plates containing chloramphenicol (20 µg/mL) and incubated at 25°C until yeast colonies emerged. Representative colonies of each distinct morphotype were purified through streak inoculation on YPD agar for further characterization. To ensure long-term preservation, yeast cultures were stored at −80°C in a broth culture supplemented with 20% (wt/vol) glycerol.

2.2 | Sanger sequencing, phylogenetic analysis and phenotypic characterization

Part of the small subunit (SSU) ribosomal RNA (rRNA) gene, the internal transcribed spacer (ITS) region, and the D1/D2 domains of the large subunit (LSU) rRNA genes were amplified and sequenced using primers NS1 and NS4 (Kurtzman & Robnett, 1998; Lachance et al., 1999; White et al., 1990), ITS1 and ITS4 (White et al., 1990), and LR0R and LR16 (Moncalvo et al., 2000; Vilgalys & Hester, 1990), respectively. The polymerase chain reaction (PCR) products were purified using QIAquick PCR columns (Qiagen) following the manufacturer's instructions. After purification, the products were sent to Macrogen for sequencing, employing the respective PCR primers. To identify the yeast species, the sequences of the ITS region were compared with those available in GenBank. This comparative analysis was conducted using the "blastn" search utility (McGinnis & Madden, 2004). To identify novel species, we used a cut-off of 97% ITS sequence identity (Lachance, 2018; Stackebrandt & Goebel, 1994; Vu et al., 2016). Furthermore, to compare the sequence identity between strains from the novel species, we also compared partial sequence of the SSU rRNA gene and the D1/D2 domains of the LSU rRNA gene (here after referred to as SSU and LSU regions, respectively). All sequences generated during the study were deposited in NCBI GenBank (Table S1). The GenBank accession numbers of the ITS, SSU, and LSU rRNA sequences are OP293325, OP293328, and OP293331 for strain ATA-11A-B (=CBS 18375^T), OP293326, OP293329, and OP293332 for strain ATA-12C-B (=CBS 18376) and OP293327, OP293330, and OP293333 for strain ATA-13E-S (=CBS 18374), respectively.

For phylogenetic analysis, only *Nakazawaea* species that contained sequences of the D1/D2 domains of the LSU rRNA gene were included. Sequences were assembled, concatenated, and aligned using the MUSCLE multiple alignment program in MEGA software version 11 (Kumar et al., 2018). The phylogenetic relationship of the novel species was determined through neighbor-joining analysis, based on the D1/D2 domains of the LSU rRNA gene, and using *Pachysolen tannophilus* as the outgroup species. For this analysis, the number of substitutions between the sequences was used as the distance metric. Confidence values were estimated from bootstrap analyses of 1000 replicates (Felsenstein, 1985). Nodes were considered supported if the bootstrap percentage was ≥50% (Hillis & Bull, 1993). The identity matrix between *Nakazawaea* species was generated with Bioedit (Hall et al., 2011).

The yeasts were subjected to morphological, physiological, and biochemical characterization under solid media using the replica-plating technique. This physiological and biochemical characterization was done using the standard methods described by Kurtzman (2011). To assess their fermentative capacity, the ability to metabolize glucose, fructose, and sucrose was examined in Durham tubes containing fermentation base media, with a final sugar concentration of 2% (wt/vol), as described by Yarrow (1998). The tubes were incubated at 25°C for a period of 14 days. For cell morphology analysis, observations were made using a Nikon Eclipse Ti2-E microscope equipped with differential interference contrast (Nikon) optics after 3 days of growth in YPD broth, incubated at 25°C. Ascospore production was assessed for each of the three isolates individually and in pairwise combinations. These assessments were conducted on dilute (1:9) V8 agar, 5% malt extract agar, yeast carbon base supplemented with 0.01% ammonium sulfate, corn meal agar, Fowell acetate agar, and glucose yeast agar. The experiments were carried out at two different temperatures, 15°C and 25°C, with a duration of up to 6 weeks. Pseudohyphae and true hyphae formation were detected using the Dalmau plate culture method, as described by Kurtzman (2011).

2.3 | Whole-genome sequencing

For genome sequencing, the strain ATA-11A-B (=CBS 18375^T) was cultured in 15 mL of YPD broth at 25°C for 72 h. Genomic DNA extraction was performed using the Qiagen Genomic-tip 20/G Kit (Qiagen), following the previously described method by Nespolo et al. (2020). The extracted DNA was quantified using the

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Invitrogen™ Qubit™ 4 Fluorometer (cat. no. Q33226; Invitrogen), and a genomic DNA library was prepared using the Illumina DNA-Prep Kit, following the manufacturer's protocol (Illumina). Subsequently, the libraries were sequenced using the Illumina NextSeq500 with a Mid-Output Kit, generating 150-bp paired-end reads, at the sequencing facility of the University of Santiago de Chile.

2.4 | Genome assembly, gene function annotation, and phylogenetic analyses

A total of 7,225,123 raw Illumina sequences were obtained, filtered, and cleaned using the trim_galore program v0.6.7 (https://github.com/FelixKrueger/TrimGalore). Out of these, 7,206,640 sequences, with both pairs preserved, were utilized for genome assembly. The de novo genome assembly was conducted using the multi-platform genome assembly pipeline (MpGAP) v3.1, employing Nextflow version 21.10.6 and Masurca version 4.0.5. To assess the quality of the assembly, Quast version 5.0.2 and Benchmarking Universal Single-Copy Orthologs (BUSCO) version 5.4.2 were employed, utilizing the Saccharomycetales_odb10 database. The assembly with the best performance (highest N50 and largest contig size [kbp]) was then annotated using the Funannotate pipeline version 1.8.14. Contigs smaller than 500 base pairs were removed, and repeated sequences were masked using default settings. Gene prediction was carried out ab initio using "Augustus," "HiQ," "GlimmerHMM," "snap," and "GeneMark." The prediction of transfer-RNAs (t-RNA) was performed using tRNAscan-SE v2.0.9, a program included in Funannotate. For annotation and functional prediction of genes we utilized InterProScan. Kyoto Encyclopedia of Genes and Genomes (KEGG) and KofamKOALA web server were used to predict the gene functions of *N. atacamensis* strain ATA-11A-B and *N. peltata* strain NRRL Y-6888. The average nucleotide identity (ANI) between *Nakazawaea* genomes was estimated from different available assemblies using OrthoANI (Lee et al., 2016).

A maximum-likelihood (ML) phylogenetic tree was constructed using a protein sequence predicted from *N. atacamensis* and other four *Nakazawaea* species (*N. peltata*, *N. holstii*, *N. ishiwadae*, and *N. ambrosiae*). The yeast species *P. tannophilus* was used as an outgroup. Ortho-Finder v2.4.1 was employed to identify orthologous protein groups among the different species. Subsequently, a total of 2422 single-copy orthologs were identified in all species and aligned using Muscle v3.8.15 (Edgar, 2004). Alignments were concatenated to produce a ML tree with RAxML v8.2.12 (-f a -x 12345 -p 12345 -# 100 -m PROTGAMMAJTT -k). The phylogenetic tree was visualized and plotted using iTOL v5.

2.5 | Microculture and fermentative phenotypic characterization

To obtain quantitative data of the different growth phases (specific growth rate, lag time, and optical density (OD) max), we generated growth curves under high throughput microcultivation conditions. The microculture assay was performed in liquid media as previously described (Nespolo et al., 2020). Briefly, strains were pre-cultivated in 200 µl 0.67% YNB medium supplemented with glucose 2% (wt/vol) for 48 h at 25°C. Each pre-inoculum (OD of 0.03–0.1) from *N. atacamensis* strains was inoculated in 200 µL of 0.67% YNB supplemented with the following carbon sources: glucose 2% (wt/vol), fructose 2% (wt/vol) and sucrose 2% (wt/vol) for 64 h incubated without agitation using a Tecan Sunrise absorbance microplate reader (Tecan). Additionally, we included environmental stressors such as ethanol 4%, 6%, and 8%, and glucose 20% (wt/vol) during 64 h. The OD was measured every 30 min using a 630 nm filter. Each experiment was carried out in triplicate. Specific growth rate, lag time, and OD max parameters were obtained for each strain using the GrowthRates software as previously described (Villarreal et al., 2022). All statistical analyses were performed using biological replicates. One-way analysis of variances (ANOVAs) were performed using GraphPad Prism 8.01 for Windows (GraphPad Software, www.graphpad.com).

Fermentations were carried out as previously described (Villarreal et al., 2022). Briefly, for each experiment, yeast cells were initially grown under constant agitation in 10 mL of synthetic wine must (SWM) for 16 h at 25°C. Next, 1×10^6 cells/mL were inoculated into 50 mL SWM (in 250 mL flasks) and the flasks were incubated in a tank fill with water at 25°C and a magnetic stirring under 125 rpm agitation (MS-M-S10 magnetic stirrer, DLAB) for 7 days. As wine fermentation control, we used *S. cerevisiae* EC1118. The experiments were carried out in triplicates. Microfermentations were weighed every day to calculate the CO_2 output. Sugar consumption, together with ethanol and glycerol production were estimated using high-performance liquid chromatography of the Bio-Rad HPX-87H column. In this way, we estimate the consumption of glucose and fructose, together with the production of glycerol and ethanol.

3 | RESULTS AND DISCUSSION

3.1 | Isolation of ethanol-tolerant yeast species from Atacama Desert flora

To identify ethanol-tolerant yeast strains from the Atacama Desert, we collected 22 samples from various tissues of native trees, including bark, bark-exuded gum, pods, and flowers. The tree species sampled included *N. chilensis* (Algarrobo), *Geoffroea decorticans* (Chañar), and *Strombocarpa tamarugo* (Tamarugo). After subjecting these samples to culture enrichment YNB media supplemented with 2% (wt/vol) glucose and 4% (vol/vol) ethanol, we selected representative colonies with distinct morphotype. In this way, a total of 91 ethanol-tolerant yeast colonies were obtained (Table S2). Among these colonies, 60 were isolated from *N. chilensis*, 10 from *S. tamarugo*, and 21 from *G. decorticans*.

Next, we amplified the ITS region and sequenced at least one colony per sample according to the size of the ITS region obtained. Based on these sequencing results, we identified 36 strains as belonging to the basidiomycetous phylum, specifically the genera

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Kwoniella (associated to Tamarugo and Chañar), *Rhodotorula* (associated to Chañar), and *Naganishia* (associated to Algarrobo). Additionally, we identified 55 strains corresponding to ascomycetous species, belonging to the genera *Zalaria* (a yeast-like fungus), *Starmerella*, and *Nakazawaea*, all of them exclusively associated to Algarrobo. To identify fermenting yeast, we conducted the Durham tube fermentation test using glucose as the carbon source. Through this test, we discovered 39 strains belonging to the genera *Nakazawaea* and *Starmerella* that exhibited the ability to ferment glucose. Notably, all of these strains were obtained from samples of *N. chilensis*. This plant is a leguminous tree known for its high levels of polysaccharides (Astudillo et al., 2000). It is also worth mentioning that the "Aloja" beverage is produced from the mashed pods of *Neltuma* species, suggesting a potential association between ethanol-tolerant yeasts and the spontaneous fermentation processes associated with this tree species. Among the strains, the majority were identified as representatives of the genus *Nakazawaea* (35 strains). However, the ITS region of these strains differed by 16%– 25% in terms of sequence identity from the *Nakazawaea* species currently described, being *N. peltata* the closest relative (Figure S1). Hence, in this study, we focus on the description of a new fermenting ascomycetous yeast species, which we have named *N. atacamensis* sp. nov.

3.2 | Novel species delineation and identification

3.2.1 | Delineation of a novel species and phylogenetic placement of *N. atacamensis* sp. nov

Three *Nakazawaea* strains from different tree samples, representing a novel anamorphic and glucose-fermenting yeast species, were isolated from *N. chilensis* samples in the Atacama Desert of Chile. Two strains of *N. atacamensis* were obtained from bark samples from two different trees (strains ATA-11A-B^T and ATA-12C-B), while the third strain was obtained from bark-exuded gum from another tree (ATA-13E-S). To assess the sequence divergence among the three *N. atacamensis* strains, we compared the ITS region, the SSU rRNA gene sequence, and the D1/D2 domains of the LSU rRNA gene. We found that the sequences of the ITS and the LSU rRNA domains were 100% identical among the three strains (Figure S2). However, in the SSU rRNA region, the strains ATA-11A-B^T and ATA-12C-B strains, both derived from bark samples, exhibited a 99.8% sequence identity compared to strain ATA-13E-S (Figure S3). Strain CBS 5808 (GenBank accession number AY366526) and the novel species have identical D1/D2 sequences. This strain was deposited in 1970 by J. Grinbergs in the CBS Yeast Collection as *Candida conglobata*, and it was isolated from tree bark in Chile. The ITS sequences of strain CBS 5808 and the novel species were also identical, showing that they represent the same yeast species. As this strain was obtained during a different sampling campaign, it was not taken into account in this study.

To determine the presence of the new *Nakazawaea* species in a phylogenetic context, we conducted a comparative analysis using the D1/D2 domains of the LSU rRNA gene from the currently accepted *Nakazawaea* species. The obtained sequences were utilized to construct a neighbor-joining tree. This tree highlighted the clustering of *N. atacamensis* with *N. pomicola*, and also indicated that *N. atacamensis* represents a novel species (Figure 1). The purpose of the neighbor-joining tree shown in Figure 1 is to demonstrate that our *Nakazawaea* strains are indicative of a novel species rather than to establish a comprehensive phylogeny of the genus. By comparing the DNA sequences of the investigated *N. atacamensis* strains with those

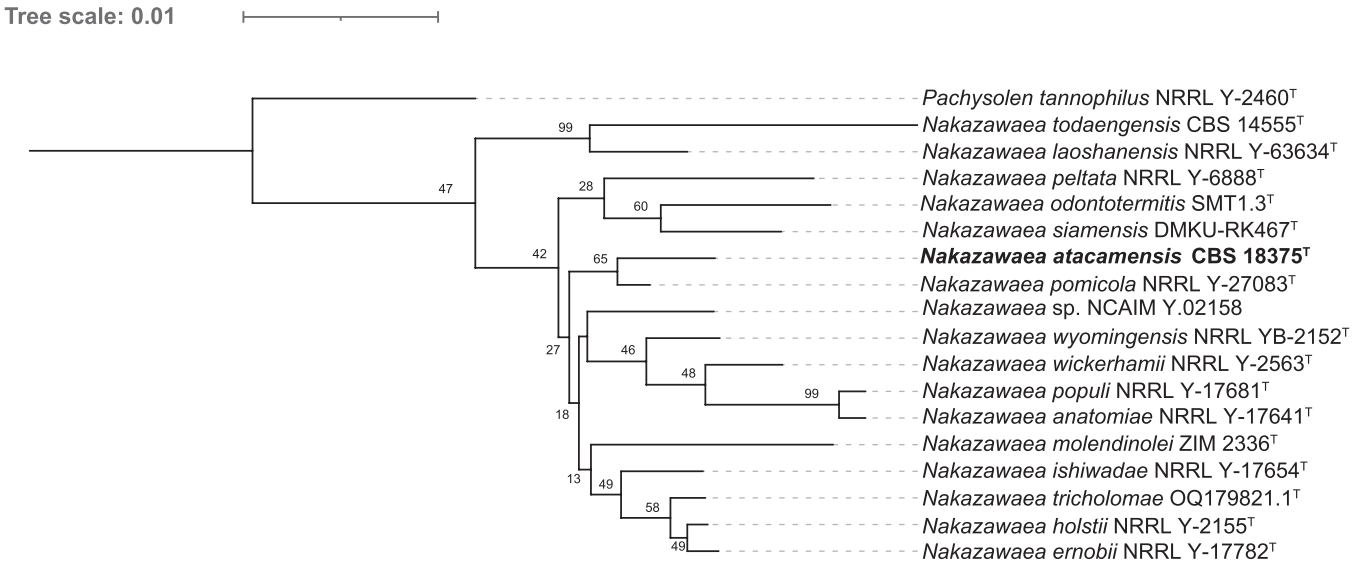

FIGURE 1 Neighbor-joining phylogram showing the placement of *Nakazawaea atacamensis* sp. nov within the genus. The tree was built based on the sequences of the D1/D2 domains of the LSU rRNA gene. The alignment was performed with MUSCLE. *Pachysolen tannophilus* was used as an outgroup. The distance metric is the number of substitutions. Bar, Substitutions per site. Bootstrap values are shown for each node. LSU, large subunit; rRNA, ribosomal RNA.

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of the genus, notable differences were observed. Specifically, *N. atacamensis* strains exhibited sequence divergences to the other species in the genus ranging from 1.3% to 5.2% in the D1/D2 domains, being *N. pomicola* the closest relative. Similarly, sequence divergence in the SSU region ranged between 1.4% and 3.5%, with *N. peltata* representing the closest relative for this marker. Ascospores were not formed in individual cultures or in mixed cultures of the strains tested. These findings provide strong evidence that the investigated strains represent a distinct anamorphic species within the *Nakazawaea* clade. Therefore, we propose the name *N. atacamensis* f. a., sp. nov. for these three strains. The mention *forma asexualis* is added as a reminder that the sexual state is not known (Lachance, 2012).

3.3 | Taxonomy

3.3.1 | *N. atacamensis* M. Araya, T. Moyano, P. Villareal, A. R. O. Santos, F. P. Díaz, A. Bustos-Jarufe, K. Urbina, R. A. Gutiérrez, C. A. Rosa, and F. A. Cubillos sp. nov

Etymology: N. atacamensis *(a. ta. ca. men'sis, NL. fem. Adj.* atacamensis, *pertaining to the Atacama Desert biome)*

The novel species displayed morphological characteristics consistent with those commonly observed in species of the genus *Nakazawaea*. After 3 days on YM agar at 25°C colonies are small, convex, grayish-white, and have an entire margin. Cells are ellipsoidal to elongate (1.1– 3.1 × 2.1–4.5 µm) and occur singly or in mother–bud pairs (Figure 2). Budding is multilateral, though predominantly at polar of cells. In Dalmau plates after 14 days at 25°C on corn meal agar, pseudohyphae and true hyphae are not observed. No ascospores or signs of conjugation were not seen on sporulation media. Fermentation of glucose, fructose, and galactose is positive, while maltose, trehalose, and sucrose are negative (Table S3). The species utilizes D-glucose, sucrose, D-galactose, α-trehalose, maltose, melezitose, cellobiose, salicin, L-rhamnose, D-xylose, L-arabinose, D-arabinose (w/s), D-ribose, ethanol, glycerol, erythritol (slow), ribitol, D-mannitol, D-glucitol, succinate, citrate, D-gluconate (slow), xylitol (slow), ethyl acetate (w/s), and N-acetyl-D-glucosamine as carbon sources. No growth occurs on inulin, raffinose, melibiose, lactose, soluble starch, L-sorbose, methanol, galactitol, myo-inositol, DL-lactate, D-glucosamine, hexadecane, acetone, and 2-propanol. Lysine is utilized as sole nitrogen source but not nitrate and nitrite. Growth in amino-acid-free medium is positive. Growth at 10°C, 20°C, and 35°C is positive. Growth on YPD agar with 10% sodium chloride and growth on 50% glucose/yeast extract (0.5%) are negative. Acid production is positive (slow). Starch-like compounds are absent. Growth in the presence of 0.01% cycloheximide is positive. Diazonium blue B color and urease reactions are negative. The holotype of *Nakazawaea atacamensis*, strain CBS 18375^T, is preserved in a metabolically inactive state at the CBS Yeast Collection of the Westerdijk Fungal Diversity Institute in Utrecht, The Netherlands. Additionally, an isotype of *N. atacamensis* has been deposited as strain RGM 3383 in the Chilean Culture Collection of Microbial Genetic Resources (CChRGM) at the Agricultural Research Institute (INIA) in Chile. The Mycobank number is MB 849680. Furthermore, 35 paratypes of *N. atacamensis* were isolated and are maintained in a metabolically inactive state at the Universidad de Santiago de Chile.

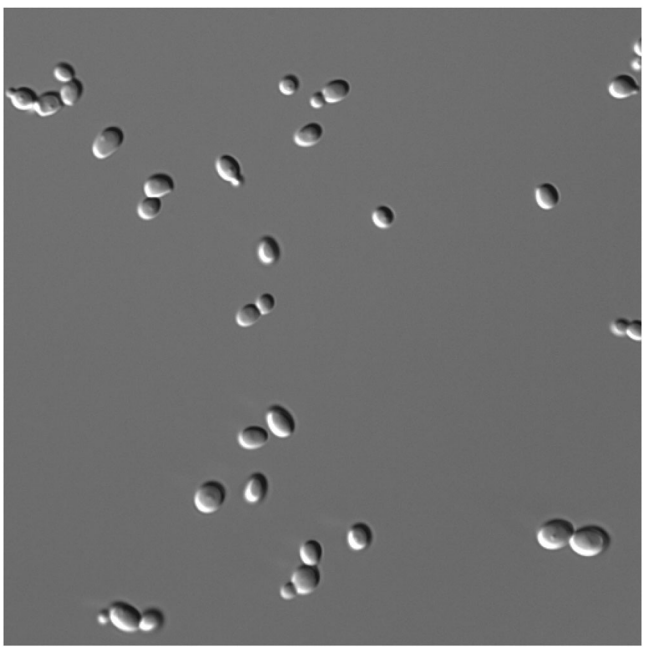

FIGURE 2 Differential interference contrast micrograph of budding cells of *Nakazawaea atacamensis* sp. nov. grown in yeast peptone dextrose broth after 3 days at 25°C. Bars: 5 µm. This image was obtained using differential interference contrast microscopy.

The novel species *N. atacamensis* exhibits distinctive phenotypic characteristics compared to other species within the genus *Nakazawaea*, as outlined in Table S3. *N. pomicola* ferment D-glucose, while *N. atacamensis* ferments both D-glucose, galactose (variable) and trehalose (variable). One notable difference is the ability of *N. atacamensis* to assimilate trehalose, while it cannot metabolize inulin and soluble starch. In contrast, *N. pomicola* is capable of assimilating soluble starch. *N. pomicola* can assimilate nitrate, whereas *N. atacamensis* does not possess this capability. These phenotypic variations highlight the distinct metabolic characteristics of *N. atacamensis* when compared to *N. pomicola*, emphasizing its unique physiological profile within the genus.

Nakazawaea is a genus of ascomycetous yeasts belonging to the class Pichiomycetes, order Alaninales, and family Pachysolenaceae (Groenewald et al., 2023). Currently, Mycobank lists 15 valid species within the genus *Nakazawaea*. These yeasts have been isolated from diverse habitats, with a predominant association with plant materials such as apples, grapes, and fermented grape musts. They have also been found in sugar cane leaves, decayed wood, and in association with wood-feeding insects like beetles (Crous et al., 2019; Kurtzman, 2011; Kurtzman & Kurtzman, 2001; Kurtzman & Robnett, 2014; Polburee et al., 2017; Tiwari et al., 2022; Yamada et al., 1994). The

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diversity of *Nakazawaea* species reflects their ability to thrive in different environments and highlights their importance in various ecological contexts. Their occurrence in plant materials and wood-associated habitats underscores their potential ecological and fermentation-related significance, including their potential involvement in the production of local fermented foods and beverages. Recent yeast surveys in mushrooms in China and termites extended the repertoire of species in the genus *Nakazawaea* (Tiwari et al., 2022) indicating a wide habitat for *Nakazawaea* species. In the present study, three strains of a novel species *N. atacamensis* were isolated from the bark and bark-exuded gum of the *N. chilensis* in the Atacama Desert, San Pedro, Chile.

3.3.2 | *N. atacamensis* whole genome sequencing

Based on the genome sequencing and assembly of the *N. atacamensis* ATA-11A-B^T, we assessed the genome complexity and quality of the novel species. Illumina sequencing yielded approximately 7.2×10^5 filtered reads, providing a sequence coverage depth of 8.8X. Seven genome assemblers were compared, and the best assembly was obtained using SPAdes/Shovill (Table S4). The resulting draft genomic of *N. atacamensis* had a 12.4 Mbp length, with an estimated GC content of 36.7%. The *N. atacamensis* assembly consisted of 115 contigs, with 42 contigs exceeding 501 bp, accounting for 99% of the assembled sequences. The largest contig had a length of 2070.2 kbp, and the N50 value of the assembly was 729,094 bp, indicating the contiguity of the assembly. The details of the sequencing results and assembled contigs can be found in Table S4.

Next, we utilize the available genomes from the *Nakazawaea* genus to generate a phylogenetic tree. The genus *Nakazawaea* currently contains four publicly available genomes: *N. ishiwadae*, *N. holstii*, *N. peltata*, and *N. ambrosiae*. The phylogenetic tree placed *N. atacamensis* together with *N. peltata* (Figure 3). In general, the level of phylogenetic resolution allowed by single genes is insufficient, while the utilization of whole genome concatenated orthologous genes represents a better approximation to resolve the phylogenetic relationship between species (Brown et al., 2001; Opulente et al., 2023; Yokono et al., 2018).

To validate species discrimination, we employed the ANI analysis across *Nakazawaea* genomes. Our examination revealed an average ANI value of 72.4% between *N. atacamensis* and the other genomes considered in this study (Table S5). Consequently, this finding supports the classification of *N. atacamensis* as a novel species, consistent with the established yeast species delineation criteria (Lachance et al., 2020). ANI serves as a robust parameter for demarcating species boundaries in yeasts using genome sequence data. Specifically, ANI values below 95%, which are indicative of distinct bacterial species, have been found also to be a good guideline for a group of well-defined yeast species (Lachance et al., 2020).

Notably, the genome size of *N. atacamensis* is comparable to that of other four *Nakazawaea* species, such as *N. ishiwadae* GDMCC 60786. Gene prediction and component analysis of the *N. atacamensis* genome using the GeneMark tool resulted in the identification of 5394 predicted genes, with a BUSCO score of 98.2%. Among these genes, 5116 protein-coding genes (95%) were annotated with InterProScan (Table S6). To facilitate the reconstruction of the molecular network from the predicted proteins, we employed KofamKOALA and assigned KEGG Orthologs. We identified 2782 genes involved in 385 pathways (Table S6). Most of the predicted genes are associated with metabolic pathways, biosynthesis of secondary metabolites, microbial metabolism in diverse environments, and biosynthesis of cofactors (Figure 4a). Given that *N. atacamensis* is a fermenting yeast, we specifically focused on carbon metabolism. Our analysis revealed the presence of 68 genes encoding enzymes involved in various carbon source metabolism pathways (Figure 4b, Table S6). These pathways include glycolysis/gluconeogenesis, pyruvate metabolism, the citrate cycle (TCA), and the pentose phosphate pathway, all of which are critical for sugar fermentation through the central carbon metabolism. Additionally, other pathways, such as glycogen biosynthesis and degradation, nucleotide sugar biosynthesis, and UDP-N-acetyl-D-glucosamine biosynthesis, may also be present in *N. atacamensis*.

Subsequently, we conducted a comparative analysis of *N. atacamensis* KEEG pathways in relation to its closest relative, *N. peltata* (Table S7). This analysis unveiled variations in gene content within several pathways, indicating potential metabolic differences between the two species (Table S8). Notably, *N. atacamensis* exhibited an increased number of genes associated with oxidative phosphorylation and the TCA cycle, likely suggesting that this yeast may rely on the electron transport chain for ATP production and energy generation via cellular respiration. These differences may

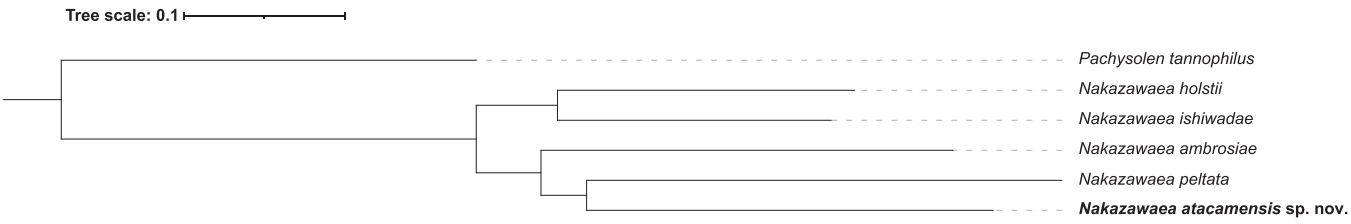

FIGURE 3 Maximum likelihood (ML) phylogenetic tree using whole-genome sequences. A concatenated alignment of 2422 single-copy orthologs was used to construct the ML tree. *Pachysolen tannophilus* was used as an outgroup. Branch lengths denote amino acid substitutions per site.

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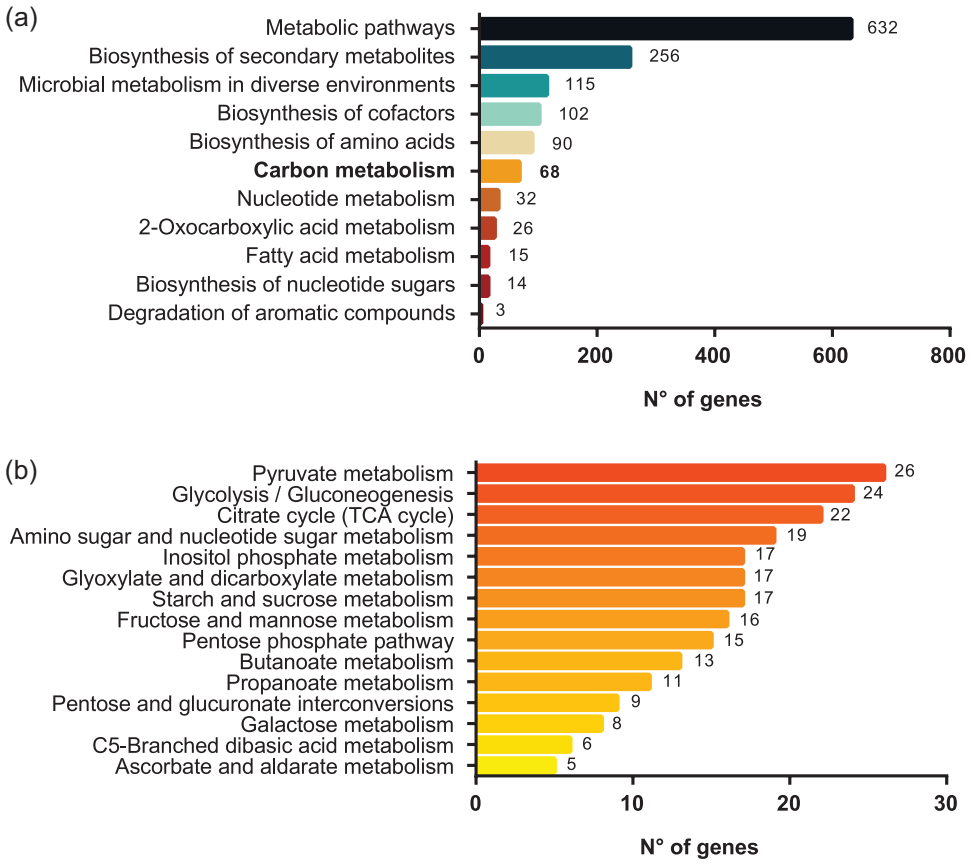

FIGURE 4 *Nakazawaea atacamensis* genome analyses. (a) KEGG distribution based on gene prediction using KofamKOALA. (b) KEGG enrichment analysis of the carbohydrate metabolism pathways. In both figures the *x*-axis depicts the number of genes on each pathway identified in the *N. atacamensis* genome. KEGG, Kyoto Encyclopedia of Genes and Genomes; TCA, tricarboxylic acid.

correlate with the hyperarid conditions where *N. atacamensis* is present, where high salt concentrations may increase gene expression in TCA cycle genes due to an increase ATP demand (Lahtvee et al., 2016; Li et al., 2021). Conversely, *N. peltata* displayed a higher number of genes involved in sulfur metabolism, suggesting an augmented capacity for sulfur assimilation, utilization, and regulation when compared to *N. atacamensis*.

3.4 | *N. atacamensis* exhibits a potential utilization in wine fermentation

To determine the potential of *N. atacamensis* for the elaboration of alcoholic beverages, we conducted initial evaluations of biomass production under microculture conditions utilizing the strain ATA-11A-B^T as representative of the species. Various carbon sources and fermentation-related conditions, including glucose and fructose utilization, as well as ethanol and high glucose concentration tolerance, were examined, with the wine *S. cerevisiae* EC1118 strain used as a comparison. Overall, *N. atacamensis* ATA-11A-B^T showed lower specific growth rates (μmax) as compared to strain EC1118 when cultured with 2% glucose or 2% fructose ($p < 0.05$, one-way ANOVA, Figure 5a, Table S9). When we evaluated growth under the sucrose disaccharide as a carbon source, we did not observe significant differences between the *N. atacamensis* and the *S. cerevisiae* strains, where strain ATA-11A-B^T exhibited a lower μmax than the *S. cerevisiae* wine strain ($p > 0.05$, one-way ANOVA, Figure 5a).

Subsequently, we subjected strain ATA-11A-B^T to ethanol and high glucose concentrations as stressors. Strain ATA-11A-B^T displayed a high tolerance to ethanol, as it was able to grow in the presence of ethanol up to 8% vol/vol (Figure 5b). However, the specific growth rates of strain ATA-11A-B^T under ethanol conditions were still lower compared to those of strain EC1118 ($p < 0.05$, one-way ANOVA). Despite this difference, the observed ethanol tolerance of strain ATA-11A-B^T highlights its potential for fermentation processes. Furthermore, the *N. atacamensis* strain exhibited a high μmax when cultured in 20% glucose, indicating its potential suitability for wine fermentation conditions. In this sense, other species from the same genus, such as *N. ishiwadae* have been previously reported to possess high ethanol tolerance and show potential for enological applications (Ruiz et al., 2019; van Wyk et al., 2020).

Based on the results obtained from the microcultures, we proceeded to conduct wine fermentations using monocultures of the *N. atacamensis* ATA-11A-B^T. To assess its fermentation capability, we measured the amount of CO_2 lost under SWM conditions,

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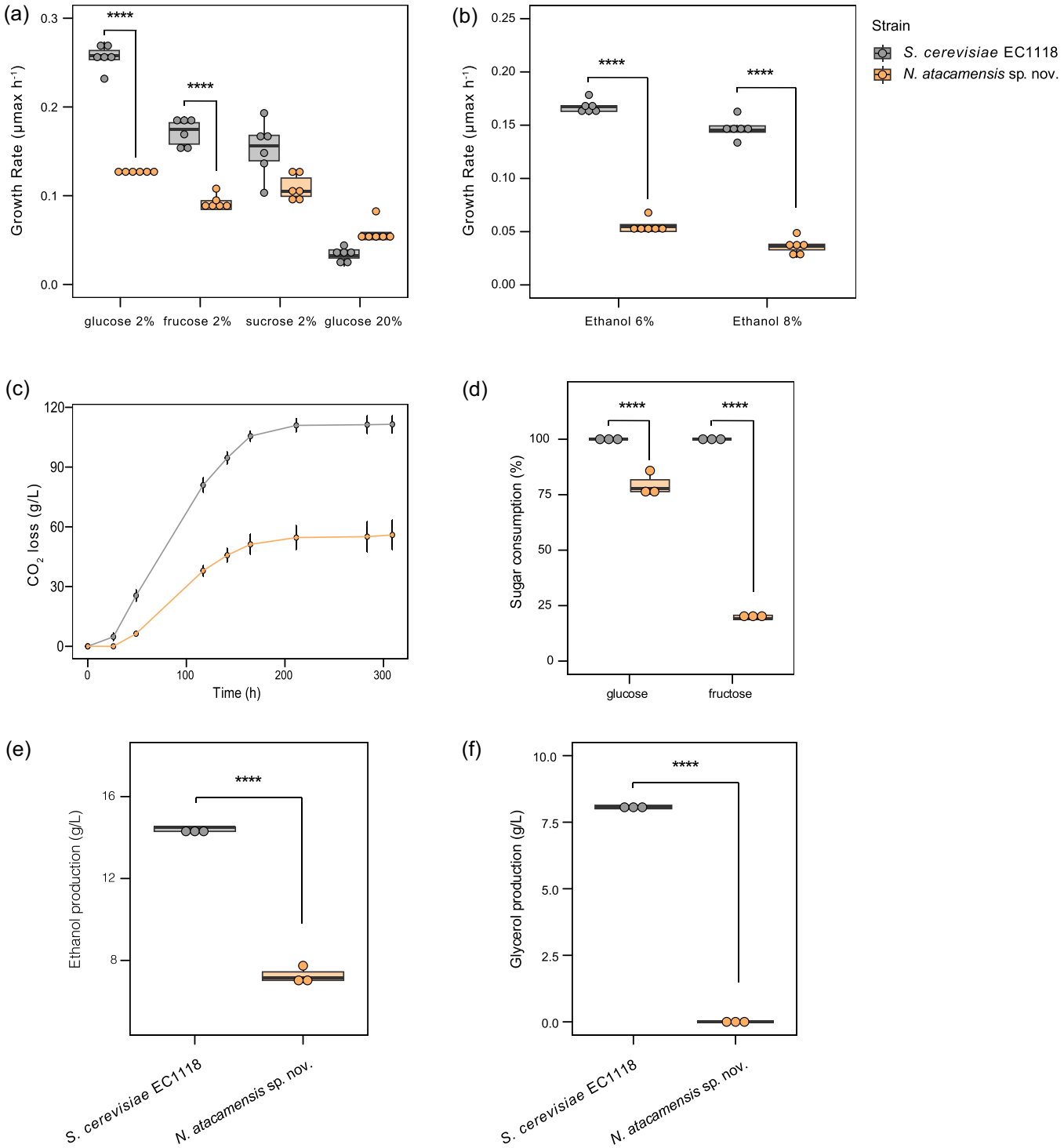

FIGURE 5 Fermentation phenotypes in *Nakazawaea atacamensis*. (a) Specific growth rate under glucose, fructose, and sucrose 2% wt/vol, and glucose 20% wt/vol as carbon sources. (b) Specific growth rates under medium supplemented with glucose 2% wt/vol and ethanol at 6% and 8% vol/vol. Fermentation results in synthetic wine must with a yeast assimilable nitrogen concentration of 300 mg/mL. (c) CO_2 loss, (d) sugar consumption, (e) ethanol production, and (f) glycerol production. EC1118 strain (gray dots) and *N. atacamensis* (orange dots). *S. cerevisiae*, *Saccharomyces cerevisiae*.

with a yeast assimilable nitrogen concentration of 300 mg/mL. The fermentation assay revealed that strain ATA-11A-B^T was capable of fermenting SWM, achieving a maximum of 75.5 g/L of CO_2 lost, which was significantly lower compared to the commercially available *S. cerevisiae* control strain ($p < 0.05$, one-way ANOVA, Figure 5c). The *N. atacamensis* ATA-11A-B^T exhibited incomplete fermentation (Figure 5d), producing ethanol levels of 7.3 ± 0.4% vol/vol (Figure 5e) and a having a residual sugar content of 126.7 g/L, comprising

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25.8 ± 7.0 g/L of glucose and 100.9 ± 2.5 g/L of fructose (Figure 5d). These results suggest that *N. atacamensis* would not represent a fructophilic yeast, such as those from the *Wickerhamiella/Starmerella*, W/S clade, and would preferentially consume glucose over fructose (Goncalves et al., 2018). In contrast, the *S. cerevisiae* EC1118 control demonstrated a higher ethanol and glycerol production (Figure 5f), reaching up to 14.4 ± 0.2% vol/vol of ethanol, with only 23.6 ± 2.4 g/L of residual fructose (Table S10). Nevertheless, it is important to note that these values are comparable to those observed with other nonconventional yeasts used in the wine industry, such as *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Starmerella bacillaris*, *Wickerhamomyces anomalus*, and *Hanseniaspora vineae* (Vejarano & Gil-Calderón, 2021). Altogether, our results indicate the prospective utilization of *N. atacamensis* in monocultures or in co-cultures with *S. cerevisiae* for wine fermentation. However, further studies are needed to support the utilization of this yeast in fermentative processes.

AUTHOR CONTRIBUTIONS

Rodrigo A. Gutiérrez and **Francisco A. Cubillos**: Conceptualization. **Macarena Araya, Pablo Villarreal, Ana R. O. Santos, Andrea Bustos-Jarufe, Kamila Urbina, Javier E. del Pino, Marizeth Groenewald**, and **Francisco A. Cubillos**: Methodology. **Macarena Araya, Pablo Villarreal**, and **Tomás Moyano**: Software. **Macarena Araya, Pablo Villarreal**, and **Tomás Moyano**: Formal analysis. **Macarena Araya, Francisca P. Díaz, Marizeth Groenewald, Rodrigo A. Gutiérrez, Carlos A. Rosa**, and **Francisco A. Cubillos**: Investigation. **Rodrigo A. Gutiérrez, Carlos A. Rosa**, and **Francisco A. Cubillos**: Resources. **Pablo Villarreal** and **Tomás Moyano**: Data curation. **Macarena Araya** and **Francisco A. Cubillos**: Writing—original draft preparation. **Pablo Villarreal, Francisca P. Díaz, Rodrigo A. Gutiérrez, Javier E. del Pino**, and **Carlos A. Rosa**: Writing—review and editing. **Rodrigo A. Gutiérrez** and Francisco A. Cubillos: Project administration. **Rodrigo A. Gutiérrez, Carlos A. Rosa**, and **Francisco A. Cubillos**: funding acquisition. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The authors acknowledge Marc-André Lachance for providing additional CBS 5808 phenotypic data. Francisco A. Cubillos acknowledges ANID FONDECYT [1220026] and ANID—Programa Iniciativa Científica Milenio NCN2021_050. Francisco A. Cubillos, Rodrigo A. Gutiérrez, Tomás Moyano, and Francisca P. Díaz are grateful to ANID—Programa Iniciativa Científica Milenio—ICN17_022, Francisca P. Díaz also acknowledges AFOREST Nucleus NCS2022_24. Rodrigo A. Gutiérrez acknowledges ANID—Programa Iniciativa Científica Milenio, Center for Genome Regulation—ICN2021_044. Macarena Araya and Javier E. del Pino acknowledge Proyecto DICYT [022343CR], Vicerrectoría de Investigación, Desarrollo e Innovación, USACH. Carlos A. Rosa was funded by "INCT Yeasts: Biodiversity, preservation and biotechnological innovation," funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil, Grant #406564/2022-1, and CNPq process numbers 0457499/2014-1, 313088/2020-9, and 408733/2021; and Fundação do Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG, process numbers APQ-01525-14, APQ-01477-13, and APQ-02552-15). Tomás Moyano is supported by ANID FONDECYT POSTDOCTORADO (Grant 3220801).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data generated or analyzed in this study is included in this article (Supporting Information files). The strains used in this study are available on request. All fastq sequences were deposited in the National Center for Biotechnology Information (NCBI) as a Sequence Read Archive under the BioProject accession number PRJNA991564 www.ncbi.nlm.nih.gov/bioproject/PRJNA991564.

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REFERENCES

Aponte-Santamaría, C., Fischer, G., Båth, P., Neutze, R., & de Groot, B. L. (2017). Temperature dependence of protein-water interactions in a gated yeast aquaporin. *Scientific Reports*, *7*, 1–14.

Astudillo, L., Schmeda-Hirschmann, G., Herrera, J. P., & Cortes, M. (2000). Proximate composition and biological activity of Chilean *Prosopis* species. *Journal of the Science of Food and Agriculture*, *80*, 567–573.

Azua-Bustos, A., Fairén, A. G., González-Silva, C., Ascaso, C., Carrizo, D., Fernández-Martínez, M. Á., Fernández-Sampedro, M., García-Descalzo, L., García-Villadangos, M., Martin-Redondo, M. P., Sánchez-García, L., Wierzchos, J., & Parro, V. (2018). Unprecedented rains decimate surface microbial communities in the hyperarid core of the Atacama Desert. *Scientific Reports*, *8*, 16706.

Azua-Bustos, A., Urrejola, C., & Vicuña, R. (2012). Life at the dry edge: Microorganisms of the Atacama Desert. *FEBS Letters*, *586*, 2939–2945.

Brown, J. R., Douady, C. J., Italia, M. J., Marshall, W. E., & Stanhope, M. J. (2001). Universal trees based on large combined protein sequence data sets. *Nature Genetics*, *28*, 281–285.

Buzzini, P., Turchetti, B., & Yurkov, A. (2018). Extremophilic yeasts: The toughest yeasts around? *Yeast*, *35*, 487–497.

Canonico, L., Solomon, M., Comitini, F., Ciani, M., & Varela, C. (2019). Volatile profile of reduced alcohol wines fermented with selected non-*Saccharomyces* yeasts under different aeration conditions. *Food Microbiology*, *84*, 103247.

Carrasco-Puga, G., Díaz, F. P., Soto, D. C., Hernández-Castro, C., Contreras-López, O., Maldonado, A., Latorre, C., & Gutiérrez, R. A. (2021). Revealing hidden plant diversity in arid environments. *Ecography*, *44*, 98–111.

Crous, P. W., Carnegie, A. J., Wingfield, M. J., Sharma, R., Mughini, G., Noordeloos, M. E., Santini, A., Shouche, Y. S., Bezerra, J. D. P., Dima, B., Guarnaccia, V., Imrefi, I., Jurjević, Ž., Knapp, D. G., Kovács, G. M., Magistà, D., Perrone, G., Rämä, T., Rebriev, Y. A., … Silva, B. D. B. (2019). Fungal planet description sheets: 868–950. *Persoonia—Molecular Phylogeny and Evolution of Fungi*, *42*, 291–473.

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Cubillos, F. A., Gibson, B., Grijalva-Vallejos, N., Krogerus, K., & Nikulin, J. (2019). Bioprospecting for brewers: Exploiting natural diversity for naturally diverse beers. *Yeast*, *36*, 383–398.

Drees, K. P., Neilson, J. W., Betancourt, J. L., Quade, J., Henderson, D. A., Pryor, B. M., & Maier, R. M. (2006). Bacterial community structure in the hyperarid core of the Atacama Desert, Chile. *Applied and Environmental Microbiology*, *72*, 7902–7908.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797.

Eizaguirre, J. I., Peris, D., Rodríguez, M. E., Lopes, C. A., De Los Ríos, P., Hittinger, C. T., & Libkind, D. (2018). Phylogeography of the wild lager-brewing ancestor (*Saccharomyces eubayanus*) in Patagonia. *Environmental Microbiology*, *20*, 3732–3743.

Eshel, G., Araus, V., Undurraga, S., Soto, D. C., Moraga, C., Montecinos, A., Moyano, T., Maldonado, J., Díaz, F. P., Varala, K., Nelson, C. W., Contreras-López, O., Pal-Gabor, H., Kraiser, T., Carrasco-Puga, G., Nilo-Poyanco, R., Zegar, C. M., Orellana, A., Montecino, M., … Gutiérrez, R. A. (2021). Plant ecological genomics at the limits of life in the Atacama Desert. *Proceedings of the National Academy of Sciences of the United States of America*, *118*, e2101177118.

Ewing, S. A., Sutter, B., Owen, J., Nishiizumi, K., Sharp, W., Cliff, S. S., Perry, K., Dietrich, W., McKay, C. P., & Amundson, R. (2006). A threshold in soil formation at earth's arid–hyperarid transition. *Geochimica et Cosmochimica Acta*, *70*, 5293–5322.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*, 783–791.

Finstad, K. M., Probst, A. J., Thomas, B. C., Andersen, G. L., Demergasso, C., Echeverría, A., Amundson, R. G., & Banfield, J. F. (2017). Microbial community structure and the persistence of cyanobacterial populations in salt crusts of the hyperarid Atacama Desert from genome-resolved metagenomics. *Frontiers in Microbiology*, *8*, 1435.

Goncalves, C., Wisecaver, J. H., Kominek, J., Oom, M. S., Leandro, M. J., Shen, X.-X., Opulente, D. A., Zhou, X., Peris, D., Kurtzman, C. P., Hittinger, C. T., Rokas, A., & Gonçalves, P. (2018). Evidence for loss and reacquisition of alcoholic fermentation in a fructophilic yeast lineage. *eLife*, *7*, e33034.

Groenewald, M., Hittinger, C. T., Bensch, K., Opulente, D. A., Shen, X. X., Li, Y., Liu, C., LaBella, A. L., Zhou, X., Limtong, S., Jindamorakot, S., Gonçalves, P., Robert, V., Wolfe, K. H., Rosa, C. A., Boekhout, T., Čadež, N., Péter, G., Sampaio, J. P., … Rokas, A. (2023). A genome-informed higher rank classification of the biotechnologically important fungal subphylum *Saccharomycotina*. *Studies in Mycology*, *105*, 1–22.

Hall, T., Biosciences, I., & Carlsbad, C. (2011). BioEdit: An important software for molecular biology. *GERF Bulletin of Biosciences*, *2*, 60–61.

Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, *42*, 182–192.

Houston, J., & Hartley, A. J. (2003). The central Andean west-slope rainshadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. *International Journal of Climatology*, *23*, 1453–1464.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Kurtzman, C. P. (2011). Nakazawaea Y. Yamada, Maeda & Mikata (1994). In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The Yeasts* (pp. 637–639). Elsevier.

Kurtzman, C., & Kurtzman, C. P. (2001). Six new anamorphic ascomycetous yeasts near. *FEMS Yeast Research*, *1*, 177–185.

Kurtzman, C. P., & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Van Leeuwenhoek*, *73*, 331–371.

Kurtzman, C. P., & Robnett, C. J. (2014). Description of *Kuraishia piskuri* fa, sp. nov., a new methanol assimilating yeast and transfer of phylogenetically related *Candida* species to the genera *Kuraishia* and *Nakazawaea* as new combinations. *FEMS Yeast Research*, *14*, 1028–1036.

Lachance, M.-A. (2012). In defense of yeast sexual life cycles: The forma asexualis—An informal proposal. *Yeast Newsletter*, *61*, 24.

Lachance, M. A. (2018). C. P. Kurtzman's evolving concepts of species, genus and higher categories. *FEMS Yeast Research*, *18*(8), foy103. https://doi.org/10.1093/femsyr/foy103

Lachance, M. A., Bowles, J. M., Starmer, W. T., & Barker, J. S. F. (1999). Kodamaea kakaduensis and *Candida tolerans*, two new ascomycetous yeast species from Australian *Hibiscus* flowers. *Canadian Journal of Microbiology*, *45*, 172–177.

Lachance, M.-A., Lee, D. K., & Hsiang, T. (2020). Delineating yeast species with genome average nucleotide identity: A calibration of ANI with haplontic, heterothallic *Metschnikowia* species. *Antonie Van Leeuwenhoek*, *113*, 2097–2106.

Lahtvee, P. J., Kumar, R., Hallström, B. M., & Nielsen, J. (2016). Adaptation to different types of stress converge on mitochondrial metabolism. *Molecular Biology of the Cell*, *27*, 2505–2514.

Lee, I., Ouk Kim, Y., Park, S. C., & Chun, J. (2016). OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *International Journal of Systematic and Evolutionary Microbiology*, *66*, 1100–1103.

Li, C., Liu, Q., Wang, Y., Yang, X., Chen, S., Zhao, Y., Wu, Y., & Li, L. (2021). Salt stress improves thermotolerance and high-temperature bioethanol production of multi-stress-tolerant *Pichia kudriavzevii* by stimulating intracellular metabolism and inhibiting oxidative damage. *Biotechnology for Biofuels*, *14*, 222.

Libkind, D., Hittinger, C. T., Valério, E., Gonçalves, C., Dover, J., Johnston, M., Gonçalves, P., & Sampaio, J. P. (2011). Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 14539–14544.

McGinnis, S., & Madden, T. L. (2004). BLAST: At the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Research*, *32*, W20–W25.

McKay, C. P., Friedmann, E. I., Gómez-Silva, B., Cáceres-Villanueva, L., Andersen, D. T., & Landheim, R. (2003). Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: Four years of observations including the El Niño of 1997-1998. *Astrobiology*, *3*, 393–406.

Moncalvo, J.-M., Lutzoni, F. M., Rehner, S. A., Johnson, J., & Vilgalys, R. (2000). Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology*, *49*, 278–305.

Morais, P. B., Pagnocca, F. C., & Rosa, C. A. (2006). Yeast communities in tropical rain forests in Brazil and other South American ecosystems. In G. Péter, & C. Rosa (Eds.), *Biodiversity and ecophysiology of yeasts* (pp. 461–484). Springer.

Mozzachiodi, S., Bai, F.-Y., Baldrian, P., Bell, G., Boundy-Mills, K., Buzzini, P., Čadež, N., Cubillos, F. A., Dashko, S., Dimitrov, R., Fisher, K. J., Gibson, B., Gouliamova, D., Greig, D., Heistinger, L., Hittinger, C. T., Jecmenica, M., Koufopanou, V., Landry, C. R., … Boynton, P. (2022). Yeasts from temperate forests. *Yeast*, *39*, 4–24.

Nespolo, R. F., Villarroel, C. A., Oporto, C. I., Tapia, S. M., Vega-Macaya, F., Urbina, K., De Chiara, M., Mozzachiodi, S., Mikhalev, E., Thompson, D., Larrondo, L. F., Saenz-Agudelo, P., Liti, G., & Cubillos, F. A. (2020). An Out-of-Patagonia migration explains the worldwide diversity and distribution of *Saccharomyces eubayanus* lineages. *PLoS Genetics*, *16*, e1008777.

10970061, 2024, 1-2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/yea.3920 by Universidad De Chile, Wiley Online Library on [28/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Opulente, D. A., LaBella, A. L., Harrison, M.-C., Wolters, J. F., Liu, C., Li, Y., Kominek, J., Steenwyk, J. L., Stoneman, H. R., VanDenAvond, J., Miller, C. R., Langdon, Q. K., Silva, M., Gonçalves, C., Ubbelohde, E. J., Li, Y., Buh, K. V., Jarzyna, M., Haase, M. A. B., … Hittinger, C. T. (2023). Genomic and ecological factors shaping specialism and generalism across an entire subphylum. *bioRxiv*. https://doi.org/10.1101/2023.06.19.545611

Pardo, O., & Pizarro, J. L. (2015). *Chile. Bebidas Fermentadas Prehispanicas*. Ediciones Parina EIRL.

Peris, D., Langdon, Q. K., Moriarty, R. V., Sylvester, K., Bontrager, M., Charron, G., Leducq, J.-B., Landry, C. R., Libkind, D., & Hittinger, C. T. (2016). Complex ancestries of lager-brewing hybrids were shaped by standing variation in the wild yeast *Saccharomyces eubayanus*. *PLoS Genetics*, *12*, e1006155.

Polburee, P., Lertwattanasakul, N., Limtong, P., Groenewald, M., & Limtong, S. (2017). *Nakazawaea todaengensis* fa, sp. nov., a yeast isolated from a peat swamp forest in Thailand. *International Journal of Systematic and Evolutionary Microbiology*, *67*, 2377–2382.

Pulschen, A. A., Rodrigues, F., Duarte, R. T. D., Araujo, G. G., Santiago, I. F., Paulino-Lima, I. G., Rosa, C. A., Kato, M. J., Pellizari, V. H., & Galante, D. (2015). UV-resistant yeasts isolated from a high-altitude volcanic area on the Atacama Desert as eukaryotic models for astrobiology. *MicrobiologyOpen*, *4*, 574–588.

Ruiz, J., Ortega, N., Martín-Santamaría, M., Acedo, A., Marquina, D., Pascual, O., Rozès, N., Zamora, F., Santos, A., & Belda, I. (2019). Occurrence and enological properties of two new non-conventional yeasts (*Nakazawaea ishiwadae* and *Lodderomyces elongisporus*) in wine fermentations. *International Journal of Food Microbiology*, *305*, 108255.

Schulze-Makuch, D., Wagner, D., Kounaves, S. P., Mangelsdorf, K., Devine, K. G., de Vera, J. P., Schmitt-Kopplin, P., Grossart, H. P., Parro, V., Kaupenjohann, M., Galy, A., Schneider, B., Airo, A., Frösler, J., Davila, A. F., Arens, F. L., Cáceres, L., Cornejo, F. S., Carrizo, D., … Zamorano, P. (2018). Transitory microbial habitat in the hyperarid Atacama Desert. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 2670–2675.

Sciammaro, L. P., Puppo, M. C., & Voget, C. (2022). Chapter 25—"Aloja": A pre-Hispanic fermented beverage from *Prosopis alba* pods. In M. C. Puppo & P. Felker (Eds.), *Prosopis as a heat tolerant nitrogen fixing desert food legume* (pp. 341–351). Academic Press.

Sciammaro, L. P., Ribotta, D. P., & Puppo, M. C. (2016). Traditional food products from *Prosopis* sp. flour. In K. Kristbergsson & J. Oliveira (Eds.), *Traditional foods: General and consumer aspects* (pp. 209–216). Springer.

Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, *44*, 846–849.

Tiwari, S., Behera, B. C., & Baghela, A. (2022). Nakazawaea odontotermitis fa, sp. nov., a novel yeast isolated from the gut of *Odontotermes horni* in India. *Archives of Microbiology*, *204*, 237.

Vejarano, R., & Gil-Calderón, A. (2021). Commercially available non-*Saccharomyces* yeasts for winemaking: Current market, advantages over *Saccharomyces*, biocompatibility, and safety. *Fermentation*, *7*, 171.

Vilgalys, R., & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, *172*, 4238–4246.

Villarreal, P., Quintrel, P. A., Olivares-Muñoz, S., Ruiz, J. J., Nespolo, R. F., & Cubillos, F. A. (2022). Identification of new ethanol-tolerant yeast strains with fermentation potential from central Patagonia. *Yeast*, *39*, 128–140.

Vu, D., Groenewald, M., Szöke, S., Cardinali, G., Eberhardt, U., Stielow, B., de Vries, M., Verkleij, G. J. M., Crous, P. W., Boekhout, T., & Robert, V. (2016). DNA barcoding analysis of more than 9000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. *Studies in Mycology*, *85*, 91–105.

Wei, X. Y., Zhu, H. Y., Song, L., Zhang, R. P., Li, A. H., Niu, Q. H., Liu, X. Z., & Bai, F. Y. (2022). Yeast diversity in the Qaidam Basin Desert in China with the description of five new yeast species. *Journal of Fungi*, *8*, 858.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols* (pp. 315–322). Academic Press.

Wierzchos, J., Cámara, B., de Los Ríos, A., Davila, A. F., Sánchez Almazo, I. M., Artieda, O., Wierzchos, K., Gómez-Silva, B., McKay, C., & Ascaso, C. (2011). Microbial colonization of Ca-sulfate crusts in the hyperarid core of the Atacama Desert: Implications for the search for life on Mars. *Geobiology*, *9*, 44–60.

van Wyk, N., Pretorius, I. S., & von Wallbrunn, C. (2020). Assessing the oenological potential of *Nakazawaea ishiwadae*, *Candida railenensis* and *Debaryomyces hansenii* strains in mixed-culture grape must fermentation with *Saccharomyces cerevisiae*. *Fermentation*, *6*, 49.

Yamada, Y., Maeda, K., & Mikata, K. (1994). The phylogenetic relationships of the hat-shaped ascospore-forming, nitrate-assimilating *Pichia* species, formerly classified in the genus *Hansenula* Sydow et Sydow, based on the partial sequences of 18S and 26S ribosomal RNAs (Saccharomycetaceae): The proposals of three new genera, Ogataea, Kuraishia, and Nakazawaea. *Bioscience, Biotechnology, and Biochemistry*, *58*, 1245–1257.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeast. In C. P. Kurtzman, & J. W. Fell (Eds.), *The Yeasts, a Taxanomy Study* (4th ed., pp. 77–100). Elsevier Publishers.

Yokono, M., Satoh, S., & Tanaka, A. (2018). Comparative analyses of whole-genome protein sequences from multiple organisms. *Scientific Reports*, *8*, 6800.

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How to cite this article: Araya, M., Villarreal, P., Moyano, T., Santos, A. R. O., Díaz, F. P., Bustos-Jarufe, A., Urbina, K., del Pino, J. E., Groenewald, M., Gutiérrez, R. A., Rosa, C. A., & Cubillos, F. A. (2024). *Nakazawaea atacamensis* f.a., sp. nov. a novel nonconventional fermentative ascomycetous yeast species from the atacama desert. *Yeast*, *41*, 52–63. https://doi.org/10.1002/yea.3920

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