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# The yeast Starmerella bacillaris (synonym Candida zemplinina) shows high genetic diversity in winemaking environments

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- 1 The yeast Candida zemplinina (Starmerella bacillaris) shows high genetic diversity in
- 2 winemaking environments
- 3 Running title: microsatellite analysis of Candida zemplinina
- 4

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#### 26 Abstract

27 The yeast Candida zemplinina (Starmerella bacillaris) is frequently isolated from grape and 28 wine environments. Its enological use in mixed fermentation with S. cerevisiae has been extensively investigated these last years, and several interesting features including low 29 30 ethanol production, fructophily, glycerol and other metabolites production, have been 31 described. In addition, molecular tools allowing the characterization of yeast populations have 32 been developed, both at the inter- and intraspecific levels. However, most of these fingerprinting methods are not compatible with population genetics or ecological studies. In 33 34 this work, we developed ten microsatellite markers for the C. zemplinina species that were 35 used for the genotyping of 163 strains from nature or various enological regions (28 36 vineyards/wineries from seven countries). We show that the genetic diversity of C. zemplinina 37 is shaped by geographical localisation and displays no evidence of domestication. Populations 38 isolated from winemaking environments are quite diverse at the genetic level: neither clonal-39 like behaviour nor specific genetic signature was associated with the different 40 vineyards/wineries. Altogether, these results suggest that C. zemplinina is not under selective 41 pressure in winemaking environments.

#### 42 Introduction

43 Yeast taxonomy is continuously evolving and new species are frequently described or their 44 phylogenetic position resolved. This is the case of *Candida zemplinina* (synonym *Starmerella* 45 bacillaris (Duarte, et al., 2012)), that was firstly described in 2003 by Matthias Sipiczki. For a 46 long time, C. zemplinina has been confounded with its sister species C. stellata that shares 47 similar ecological niches, particularly grape and wine environments (Sipiczki, 2004, Sipiczki, et al., 2005, Csoma & Sipiczki, 2008, Duarte, et al., 2012). Indeed, C. zemplinina is almost 48 systematically found in grape must, whatever the region or the grape variety, usually at 49 relatively high population level of 10<sup>e4</sup>-10<sup>e6</sup> cells/ml (Nisiotou, et al., 2007, Zott, et al., 2008, 50 Tristezza, et al., 2013, Pfliegler, et al., 2014, Sun, et al., 2014). Like Saccharomyces species 51 52 and Hanseniaspora uvarum, C. zemplinina is also detected at lower levels on winery surfaces 53 prior to harvest, and may be the source of repeated inoculation in successive batches 54 (Bokulich, et al., 2013). Then, its presence during subsequent grape fermentation is frequently 55 reported, even if S. cerevisiae dominates yeast microbiota at that point (Nisiotou, et al., 2007, 56 Cordero-Bueso, et al., 2013). Indeed, only some Saccharomyces species are able to complete 57 alcoholic fermentation (AF) in enological conditions (i.e. to consume all sugars present in 58 grape must), explaining why C. zemplinina and other non-Saccharomyces species are less 59 identified during AF. However, some NS species, of which C. zemplinina, can be isolated 60 even at the end of AF, which is congruent with the fact that some strains can produce and 61 tolerate relatively high levels of ethanol (Rantsiou, et al., 2012). C. zemplinina occurrence is 62 particularly high within sweet wines whose musts have high initial sugar concentration 63 (Sipiczki, 2003, Urso, et al., 2008, Tofalo, et al., 2009, Magyar & Toth, 2011, Rantsiou, et 64 al., 2012, Rantsiou, et al., 2013). C. zemplinina is more rarely isolated from other substrates, such as local fermentations, fruits (usually rotting fruits), fruit-associated insects or soil 65 66 (Nielsen, et al., 2005, Stamps, et al., 2012). This suggests that C. zemplinina primary 67 ecological reservoir is alcoholic fermentation of fruit juice and particularly of grapevine, with 68 occasional colonisation of other favourable niches.

These last 10 years, several authors have highlighted the enological potentials of *C. zemplinina* species (Ciani & Comitini, 2015). In mixed fermentation with *S. cerevisiae* (necessary to complete AF), it produces wine with reduced ethanol levels (Di Maio, *et al.*, 2012, Bely, *et al.*, 2013, Giaramida, *et al.*, 2013, Englezos, *et al.*, 2015). Such modifications of sugar/ethanol yield may be due, at least partially, to an increased content of glycerol (Di Maio, *et al.*, 2012, Giaramida, *et al.*, 2013, Zara, *et al.*, 2014). This feature is particularly interesting since global warming and the evolution of viticulture practices have led to grape 76 must with increased sugar content and thus increased potential ethanol content. C. zemplinina 77 species has also been investigated for its fructophilic character (Magyar & Toth, 2011, Tofalo, et al., 2012, Englezos, et al., 2015), an interesting characteristic in winemaking. Other 78 79 promising metabolic features include modification of anthocyanin profiles (Mangani, et al., 80 2011), higher level of some terpenes and lactones (Sadoudi, et al., 2012), the release of 81 mannoproteins (Domizio, et al., 2014), malic acid metabolization (Tofalo, et al., 2012), or the 82 production of some organic acid (Magyar, et al., 2014). Finally, mixed cultures including S. 83 cerevisiae and C. zemplinina were associated with increased production of some aromatic 84 compounds (Andorrà, et al., 2012), and tools are now developed in order to follow thoroughly 85 the different populations in mixed cultures (Wang, et al., 2014). However, sensory evaluation of mixed cultures were not fully satisfying (Bely, et al., 2013). Thus, C. zemplinina species 86 87 appears as an interesting non-Saccharomyces (NS) yeast in winemaking to limit the 88 production of some metabolites (ethanol), or to increase the production of other ones (glycerol, mannoproteins, etc.). However, several efforts must be performed in order to 89 90 improve the species and propose strains with neutral impact on the organoleptic properties of 91 wine. To date, C. zemplinina improvement remains difficult as the biology of the species is 92 poorly known and due to the limited amount of molecular methods developed for non-93 Saccharomyces.

94 At the interspecific level, some tools are available to characterize yeast populations during 95 alcoholic fermentation process. Besides culture-dependent methods, molecular methods can 96 be used to discriminate the different species in enological environments and to monitor their 97 growth, such as PCR-DGGE (Urso, et al., 2008), quantitative PCR (Andorra, et al., 2010, 98 Zott, et al., 2010) or High-Throughput Sequencing that has been recently proposed (Bokulich, 99 et al., 2013). These tools are particularly valuable to describe yeast dynamics in various AF 100 context, but also to study the impact of oenological practices, the consequences of farming 101 practises, etc. (Andorrà, et al., 2008, Milanovic, et al., 2013, Albertin, et al., 2014, Martins, et 102 al., 2014). At the intra-specific level, few methods are described. The mitochondrial genome 103 of C. zemplinina has been fully sequenced and intra-specific variations were described within 104 intronic sequences, allowing the description of two different mitochondrial patterns 105 (Pramateftaki, et al., 2008). RAPD-PCR fingerprinting (Tofalo, et al., 2012, Pfliegler, et al., 106 2014) and tandem repeat-tRNA (TRtRNA) PCR method (Barquet, et al., 2012) allow higher 107 intra-specific discrimination, yet do not allow accurate population genetics or ecological 108 studies. Multi-locus microsatellite typing has been successfully developed for yeast species of 109 enological interest, such as Saccharomyces cerevisiae (Legras, et al., 2007), S. uvarum

110 (Masneuf-Pomarede, et al., 2007), Torulaspora delbrueckii (Albertin, et al., 2014) or the spoilage species Brettanomyces bruxellensis (Albertin, et al., 2014). Microsatellite analysis 111 112 provided new insights into the genetic variability and population structure of wine yeasts, and 113 also provided valuable data regarding the life-cycle of the species (Albertin, et al., 2014). In 114 this work, we developed 10 microsatellite markers for the C. zemplinina species that were 115 used for the genotyping of 163 strains from nature and various winemaking regions. We show 116 that the genetic diversity of *C. zemplinina* is shaped by geographical localisation and displays 117 no evidence of domestication. Populations isolated from winemaking environments are quite 118 diverse and no specific genetic signature were associated with the different 119 vineyards/wineries.

#### 120 Material and Methods

#### 121 Yeast strains and culture conditions

122 163 strains of C. zemplinina were sampled from different collections (Table 1) and were 123 mostly isolated from different vineyards or wineries in Europe (France, Greece, Hungary, 124 Italy, Spain, Switzerland), and New Zealand. Six strains from nature were included (11-479, 11-60, 11-9, UWOPS 07-402.2, UWOPS83-775.2, UWOPS 91-743.1). In addition, the type 125 strain of *C. stellata* CBS 157<sup>T</sup> was used to test the specificity of the microsatellites markers. 126 All strains were grown at 24°C in YPD medium containing 1% yeast extract (w/v, Difco 127 128 Laboratories, Detroit, MI), 1% Bacto peptone (w/v, Difco), and 6% glucose (w/v), 129 supplemented or not with 2% agar (w/v).

130

#### 131 Genomic DNA extraction and species assessment

132 For genomic DNA extraction, cells grown on YPD medium were lysed using a FastPrep-24 133 instrument (MP Biomedicals, Illkirch, France): 100 µL of glass beads (acid-washed, 425-600 134 μm, Sigma, Lyon, France) were added to cells pellet as well as 300μl of Nuclei Lysis solution 135 (Wizard Genomic DNA purification Kit, Promega). Cells were crushed through 2 cycles of 136 20s (max. speed). Subsequent DNA extraction was performed with the Wizard Genomic 137 DNA purification Kit (Promega) following the manufacturer's protocol. A second step of 138 protein precipitation solution, as well as subsequent precipitation using isopropanol and 139 ethanol was performed in order to ensure high purity DNA extraction.

For the rapid genotyping of *B. bruxellensis* strains, we used a punch-based method using FTA CloneSaver card (Whatman, BioScience, USA). Eight  $\mu$ l of cells grown on YPD medium were loaded on a CloneSaver card, then before PCR, 2.0-mm disks were punched, washed twice with 50 $\mu$ l of TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) and once with 50 $\mu$ l of ultrapure water.

For each *C. zemplinina* strain, a PCR-RFLP method (ITS-5.8S rDNA amplification followed by *Mbo*I restriction) was used as described by Sipiczki (2004) in order to confirm species identity and exclude strains from the sister species *C. stellata*.

148

#### 149 Genome sequencing and *de novo* assembly of the *C. zemplinina* type strain CBS9494

150 A draft genomic sequence was produced using Ion Torrent technology. Briefly, genomic

151 library of CBS 9494 was produced using the Ion Xpress<sup>™</sup> Plus Fragment Library Kit (Life

152 Technologies, Carlsbad, USA), with an enzymatic shearing of 10min at 37°C. DNA was

153 sequenced on an Ion Torrent PGM (Life Technologies, Carlsbad, USA). After trimming on

quality threshold (Phred-type quality score of Q20,  $Q_{Phred}=20$ ) and length threshold (50pb) using CLC Genomics Workbench (CLC bio, Boston, USA), a total of 5,698,579 reads (mean sequence: 200pb) were used for de novo assembly using Newbler (454 Life Sciences). The 268 assembled contigs (mean: 108,648pb, max: 649,352pb) formed a 9,3Mb sequence assembly for an estimated genome size of 9.8Mb (Sipiczki, 2004).

159

#### 160 Microsatellite loci identification and primers design

161 Trinucleotide repeats were searched within the *de-novo* genome assembly of the type strain 162 CBS 9494. In order to exclude possible telomeric and subtelomeric repeats, we did not 163 considered microsatellites located within 3Kb of the 5'-end and 3'-end of the contigs. Primers 164 designed using the 'Design primers' tool the SGD website were on 165 (http://www.yeastgenome.org/cgi-bin/web-primer). To reduce the cost associated with 166 primers fluorescent labelling, the forward primers were tailed on 5'-end with M13 sequence 167 (19nt) as described by Schuelke, 2000 (Schuelke, 2000), and universal M13 primers labelled 168 with different fluorescent dyes were added (see below). Amplified fragment sizes varied from 169 101 to 361 bp, allowing subsequent multiplexing of the amplicons (Table 2).

170

#### 171 Microsatellites amplification

172 PCR reactions were performed in a final volume of  $15\mu$ l containing one washed punch from 173 FTA CloneSaver card, 0.05  $\mu$ M of forward primer, 0.5  $\mu$ M of reverse primer and labelled 174 primer, 1X Taq-&GO (MP Biomedicals, Illkirch, France). Universal M13 primers were 175 labelled with either FAM-, HEX-, PET- or NED-fluorescent dyes (Eurofins MWG Operon, 176 Les Ulis, France).

Touch-down PCR were carried out using iCycler (Biorad, Hercules, CA, USA) thermal
cycler. The program encompassed an initial denaturation step of 1 min at 94°C followed by

179 10 cycles of 30 s at 94°C, 30 s at Tm+10°C (followed by a 1°C decrease per cycle until Tm is

reached) and 30 s at 72°C, then 20 cycles of 30 s at 94°C, 30 s at Tm and 30 s at 72°C, and a

181 final extension step of 2 min at  $72^{\circ}$ C.

Amplicons were initially analysed by a microchip electrophoresis system (MultiNA, Shimadzu) and the optimal conditions for PCR amplifications were assessed. Then, the sizes of the amplified fragments were measured on an ABI3730 DNA analyzer (Applied Biosystems). For that purpose, PCR amplicons were diluted (1800-fold for FAM, 600-fold for HEX, 1200-fold for PET and 1800-fold for NED-labelled amplicons respectively) and multiplexed in formamide. LIZ 600 molecular marker (ABI GeneScan 600 LIZ Size Standard, Applied Biosystem) was 100-fold diluted and added for each multiplex. Before loading,
diluted amplicons were heated 4 min at 94°C. Allele size was recorded using GeneMarker
Demo software V2.4.0 (SoftGenetics).

191

#### 192 Data analysis

Microsatellite analysis was used to investigate the genetic relationships between strains. A dendrogram was built using Euclidean distance and Neighbor-Joining's clustering, by means of R (R Development Core Team, 2010) and package phyclust version 0.1-14 (Chen & Dorman, 2013). In order to assess the robustness of the tree nodes, multiscale bootstrap resampling associated with an approximately unbiased test (Shimodaira, 2002) was performed by means of R and the pvclust package v1.2-2 (Suzuki & Shimodaira, 2006, R Development Core Team, 2010).

In addition to dendrogram drawing, the software STRUCTURE (V2.3.4) was used to delineate clusters of individuals on the basis of their microsatellite genotypes using a Bayesian approach (Pritchard, et al., 2000). The parameters were as followed: 10000 Burn-in period, 1000 Repetitions. Models with number of populations (K) ranging from K=3 to K=20 were tested, and models with and without admixture gave similar results (the model with no admixture was thus conserved for the graphical representation of the population).

206 To test for population differentiation, analysis of molecular variance (AMOVA) was 207 performed by means of the pegas package (Paradis, 2010) with n=1000 permutations. We 208 tested whether the genetic distance was significantly explained by geographical localisation 209 (i.e. the country of isolation was used as grouping factor) or substrate origin ('Enology' 210 versus 'Wild' origins). The relationship between genetic distance and geography was 211 furthermore confirmed by Mantel's test (Mantel, 1967) using ade4 package (Chessel, et al., 212 2004). Mantel's test allows correlating two distance matrices, in that case we used the genetic 213 distance matrix computed from microsatellite data, and a kilometric distance matrix 214 (computed using latitude and longitude of strain location).

#### 216 **Results**

#### 217 Development of microsatellite markers for *Candida zemplinina*

- Ion Torrent technology was used to produce a raw sequence (268 contigs) of the genome
  sequence of CBS 9494T, the type strain of *C. zemplinina*. Microsatellite loci were searched
- within this draft genome, and we considered dinucleotide and trinucleotide repeats that were not located within the 5'-end and 3'-end of the contigs, in order to exclude possible telomeric
- 222 or subtelomeric positions. Primers were designed to amplify ten microsatellite loci (Table 2),
- 223 none of them being located in coding sequence.
- The amplicons were separated using a microchip electrophoresis system (MultiNA), and the optimal conditions for microsatellites amplifications were assessed on a panel of twenty strains of *C. zemplinina* (data not shown). After optimisation, the microsatellites markers were tested on *C. stellata*, the sister species of *C. zemplinina*. No amplification was observed for
- CBS 157T, indicating that the microsatellite markers developed were specific of *C. zemplinina* species.
- 230 The 10 microsatellites markers were then used to genotype 157 C. zemplinina strains isolated 231 from various oenological regions (Figure 1, Table 1). Six strains from non-enological 232 environments (soil, insect, other fruits) were also genotyped: 11-479, 11-60, 11-9, UWOPS 233 07-402.2, UWOPS83-775.2, UWOPS 91-743.1. All microsatellites were polymorphic, with 3 234 different alleles for CZ13 and up to 19 alleles for CZ54 (Table 2). Over the 163 strains, 121 235 different genotypes were observed, confirming the discriminant power of microsatellite 236 analysis. Interestingly, only one strain displayed heterozygosity for two loci (CZ15, CZ59), 237 while all other 162 strains showed only one allele per locus.
- 238

#### 239 Establishment of the genetic relationships between C. zemplinina strains

240 The genetic relationships between the 163 strains of C. zemplinina were further examined 241 using the Euclidean distance and Neighbor-Joining clustering. The resulting dendrogram tree 242 showed 4 main clusters: the first one included most Spanish strains (9 upon 15) as well as 243 many French strains (23 upon 83) and was quite robust (bootstrap value of 93). One group 244 contained most Italian (11/19) and Greek (10/21) strains, while another one harboured several 245 French strains (33 upon 83), these two groups being less robust (bootstrap value of 58). 246 Finally, the last group, although robust (bootstrap value of 90), contained strains from France 247 (11) as well as Greece (6), Spain (4) or a few other countries.

Another complementary analysis, using Bayesian approach, was applied to assess the significance of these four clusters. STRUCTURE found an optimum of K=4 populations that

captured most of the genetic structure of *C. zemplinina* species, and was congruent with the
dendrogram tree. In particular, the two groups with moderate bootstrap values were clearly
related to two distinct ancestral groups as determined by STRUCTURE (Figure 1).

253 In order to definitively determine whether, and to what extent, the genetic variation of 254 C. zemplinina was related to geographical origin, an analysis of molecular variance 255 (AMOVA) was performed. We used the country of isolation as grouping factor. The geographical origin was significantly related to genetic data (pvalue  $<<10^{e-6}$ ) and explained 256 29.71% of the total variation of the microsatellite dataset. The relationship between genetic 257 258 distance and kilometric distance between strains was also confirmed by Mantel's test 259 (pval=0.013, Ho= incongruence of genetic/geographic matrices). This indicated that 260 geographical origin shaped significantly, yet not completely, the genetic diversity of 261 *C. zemplinina* species. By contrast, the substrate origin appeared to impact poorly the genetic 262 diversity of the species: the 6 strains from non-enological environments (11-479, 11-60, 11-9, 263 UWOPS 07-402.2, UWOPS83-775.2, UWOPS 91-743.1) were distributed throughout the 264 dendrogram tree, while AMOVA using ecosystem ('Enology' versus 'Wild' origins) as 265 grouping factors was non-significant. Although the 'wild' panel was low (only 6 strains), 266 these data suggested that substrate origin did not significantly shaped the genetic diversity of 267 C. zemplinina species.

268

#### 269 **Population diversity in oenological conditions**

C. zemplinina occurrence is particularly high within sweet wines. Indeed, our collection included several strains isolated from high sugar grape musts (38 strains), as well as 112 strains from non-sweet wines. We thus performed an AMOVA using sugar concentration (sweet musts/wines versus non-sweet musts/wines) as grouping factors. The AMOVA was non-significant, indicating that the sugar concentration of the medium was not related to genetic diversity.

We studied strains from different European vineyards/wineries (France, Greece, Hungary, Italy, Spain, and Switzerland) and from New Zealand. Strains isolated from the same vineyard/winery usually displayed quite different genotypes and were frequently distributed throughout the dendrogram tree (Figure 2). For example, 10 strains were isolated from Winery 8 near Bordeaux (different samples and different years). Nine upon ten different genotypes were evidenced (L0629 and L0653 sharing the same genotype), belonging to all four groups (Figure 2), suggesting that no specific genotype showed persistence within a 283 given winery across tanks and vintages. The absence of 'genetic signature' at the 284 vineyard/winery level was observed for most of the vineyards/wineries we tested (Figure 2).

285 In addition, some strains were also isolated from one unique sample (see Table 1), as it is the 286 case of the NZ strains (NZ2, NZ6, NZ8, NZ11 and NZ12), all coming from one unique 287 harvest of Chardonnay fermenting must (Sample 10). A total of 71 strains coming from 19 288 unique samples were genotyped. Few clonal populations were evidenced (samples 7, 9, 14). 289 In some cases, strains isolated from one unique sample clustered in the same group as 290 evidenced on the dendrogram tree (samples 7, 9, 11, 13, 14, 15 and 18). However, in most 291 cases, strains isolated from one unique sample clustered in two different groups (samples 1, 2, 292 3, 5, 8, 16, 17 and 19) or in three different groups (samples 4, 10 and 12). An extreme case 293 was for sample 6 for which 16 strains were isolated, showing 15 different genotypes 294 distributed on the four clusters of the dendrogram tree. Globally these data indicated that 295 C. zemplinina populations associated with winemaking were not clonal populations, and that 296 no specific genetic signature were associated with the different samples and 297 vineyards/wineries.

#### 298 **Discussion**

# 299 Microsatellite genotyping, a discriminant tool for population genetics studies of C. 300 zemplinina

301 Non-Saccharomyces yeast species are currently studied for their potential oenological interest 302 (Jolly, et al., 2014). Thus, some tools are being developed to allow their genetic 303 characterization, at the interspecific level but also at the intra-specific level in order to 304 discriminate and evaluate different strains of the same species. Few methods were described 305 for C. zemplinina species: Pfliegler et al. (2014) observed moderate diversity using PCR-306 fingerprinting methods with 14 patterns for 35 tested strains. Tofalo et al. obtained quite 307 discriminant patterns from 36 strains and suggested an important genetic heterogeneity of the 308 C. zemplinina species. Tandem repeat-tRNA (TRtRNA) PCR method also appeared as a 309 promising discriminant approach (Barquet, et al., 2012). However, all these methods are 310 unsuitable for population genetics, and may yield different dendrograms and clusters 311 (Pfliegler, et al., 2014).

312 The microsatellite tool is extremely popular for population and ecological studies of many 313 species. In yeast, it has been successfully applied to several wine species: S. cerevisiae 314 (Legras, et al., 2005, Legras, et al., 2007), S. uvarum (Masneuf-Pomarede, et al., 2007, 315 Zhang, et al., 2015), Torulaspora delbrueckii (Albertin, et al., 2014), Brettanomyces 316 bruxellensis (Albertin, et al., 2014). One main advantage of the microsatellite tool lies in its 317 portability, meaning that genotyping across different laboratories can be compared. This is not 318 the case for fingerprinting approaches, which are prone to interlaboratory variation. In this 319 work, ten microsatellites markers were developed and successfully applied to 163 strains of 320 C. zemplinina. Microsatellite genotyping appeared highly discriminant, with 121 different 321 patterns. Moreover, population structure inferred from microsatellite data appeared reliable as 322 classical clustering and Bayesian approaches yielded similar results. The microsatellite tool 323 will be of interest for subsequent ecological analysis, and also in applied research for the 324 checking of strain implantation in mixed-cultures for example.

325

#### 326 *Candida zemplinina* species shows no evidence for domestication

The genetic diversity of *C. zemplinina* species showed no specific clustering depending on the substrate origin of the strains ('Enology' versus 'Wild' origins). Although the number of 'wild' strains was relatively low (6 strains), no specific clustering was observed as these wild strains sorted in two of the four clusters on the dendrogram tree. In addition, as *C. zemplinina* is known to be particularly associated with high sugar musts or wines, we tested whether 332 initial sugar concentration could be related to genotype selection. We did not find any 333 relationship between the genetic diversity and sweet/non-sweet wines, suggesting that high 334 sugar concentration in winemaking has no impact on C. zemplinina selection and adaptation. 335 Indeed, C. zemplinina species showed no evidence for domestication event. By contrast, 336 previous studies using microsatellite data demonstrated the domestication of other yeasts for 337 winemaking: S. cerevisiae was the first wine yeast shown to be domesticated for human 338 application, including winemaking, bakery, brewery, etc. (Legras, et al., 2005, Legras, et al., 339 2007). Approaches using comparative genomics later confirmed these results (Liti, et al., 340 2009) and S. cerevisiae was established as a relevant model of domesticated microorganism 341 (Sicard & Legras, 2011). Among the Saccharomyces sensu stricto complex, S. uvarum, 342 sometimes used in winemaking and cidermaking, seems to be selected for human application 343 (Almeida, et al., 2014), and the presence of introgressed genome portion could be a molecular 344 mechanisms underlying domestication in that species. Recently, a non-Saccharomyces 345 species, related to wine, was also studied using microsatellite markers. T. delbrueckii has been 346 associated with winemaking and other bioprocesses (bakery, dairy products, etc.) for decades 347 (Albertin, et al., 2014). The genetic diversity of T. delbrueckii was congruent with two 348 domestication events associated with winemaking from one hand, and other bioprocesses on 349 the other hand. Indeed, winemaking and other human application have strongly shaped the 350 genetic diversity of several yeasts, including Saccharomyces and non-Saccharomyces ones. 351 By contrast, C. zemplinina shows no evidence for such domestication events. Two opposite 352 hypotheses could be congruent with those results: first, C. zemplinina could be a fully 353 domesticated species, meaning fully associated with oenology, without actual wild relatives. 354 In that case, the few strains isolated from natural environments would come from dispersion 355 from nearby enological environments. However, one would expect such yeast populations to 356 be selected in winemaking environments, and thus to be more clonal-like. The second 357 hypothesis is that C. zemplinina is not a domesticated species. This last hypothesis would be 358 congruent with the fact that no clonal behaviour is observed, that no genetic evidence of 359 domestication is found and, conversely, that geographical origin significantly shaped the 360 genetic diversity of the species (which is expected for non-domesticated species). Even if C. 361 zemplinina possess interesting oenological properties (low ethanol production, fructophily, 362 high glycerol production, etc.), more efforts should be put into the study of physiology and 363 metabolism of this non-Saccharomyces especially in relation to its impact on the organoleptic 364 characteristics of the wine.

#### 366 The life cycle of *Candida zemplinina*:

367 In addition to population structure, microsatellite analysis may be useful to raise the curtain 368 on the life-cycle of the species (Paolocci, et al., 2006, Albertin, et al., 2014). Here, we showed 369 that all 163 strains bar one showed only one allele per locus. Under the assumption of a 370 diploid species, almost complete homozygosity could be explained by a high level of 371 sporulation leading to fully homozygous diploid representative. However, no ascospores 372 formation was evidenced, even after several weeks of incubation on traditional sporulation 373 medium (Sipiczki, 2003). Indeed, since C. zemplinina strains showed no evidence of 374 sporulation ability, it can be hypothesized that this species has a mostly haploid life-cycle, 375 with essentially haploid (homozygous) individuals and rare diploid (heterozygous) 376 representative. Further experiments, like various assays of sporulation and breeding between 377 strains will elucidate definitively the life-cycle of C. zemplinina species.

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- 546 Tables
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#### 548 Table 1. Origin of *Candida zemplinina* and *C. stellata* strains used in this study.

<sup>a</sup> Strains having the same sample code in brackets are strains isolated from the same sample.

<sup>b</sup> CBS-KNAW: Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, 550 551 institute of the Royal Netherlands Academy of Arts and Sciences (Koninklijke Nederlandse 552 Akademie van Wetenschappen), Utrecht, the Netherlands; CRB Oeno: Centre de Ressources 553 Biologiques Œnologie (Isabelle Masneuf-Pomarede), Villenave d'Ornon, France; CRPR: 554 Centre de Recherche Pernod-Ricard (Benoit Colonna-Ceccaldi), Creteil, France; Debrecen: 555 University of Debrecen (Matthias Sipiczki), Hungary; DEMETER: Hellenic Agricultural 556 Organisation (Aspasia Nisiotou), Wine Institute of Athens, Greece; DISAFA: University of 557 Torino (Luca Cocolin), Italy; Foggia: University of Foggia (Giuseppe Spano), Italy; IUVV: 558 Institut Universitaire de la Vigne et du Vin "Jules Guyot" (Hervé Alexandre), Dijon, France; 559 **ISVV**: Institut des Sciences de la Vigne et du Vin (Marina Bely), Villenave d'Ornon, France; URV: Universitat Rovira i Virgili (Albert Mas), Tarragona, Spain; UWOPS: Culture 560 561 collection of the University of Western Ontario (Marc-André Lachance), Department of 562 Biology (formerly Plant Sciences), London, Canada.

|               |        |                     |                           | Winery   | Collection/ |                        |
|---------------|--------|---------------------|---------------------------|----------|-------------|------------------------|
| Species       | Strain | Geographical origin | Substrate                 | (sample) | Laboratory  | Reference              |
|               |        |                     | Enology - fermenting      |          |             |                        |
|               |        |                     | sweet botrytized musts,   |          |             |                        |
| C. zemplinina | 10-373 | Hungary, Tolcsva    | 2001                      | Winery 1 | Debrecen    | Sipiczki, 2003         |
|               |        |                     | Enology - fermenting      |          |             |                        |
|               |        |                     | sweet botrytized musts,   |          |             |                        |
| C. zemplinina | 10-374 | Hungary, Tolcsva    | 2001                      | Winery 1 | Debrecen    | Sipiczki, 2003         |
|               |        |                     | Enology - fermenting      |          |             |                        |
|               |        |                     | sweet botrytized musts,   |          |             |                        |
| C. zemplinina | 10-375 | Hungary, Tolcsva    | 2001                      | Winery 1 | Debrecen    | Sipiczki, 2003         |
|               |        |                     | Enology - fermenting must |          |             |                        |
| C. zemplinina | 10C    | Italy, San Severo   | (Uva di Troia), 2011      |          | Foggia      |                        |
| C. zemplinina | 11-1   | Spain, Almeria      | Enology - grape must      |          | Debrecen    | Pfliegler et al., 2014 |
|               |        |                     | Enology - botrytized      |          |             |                        |
| C. zemplinina | 11-101 | Hungary, Tarcal     | grape, 2002               |          | Debrecen    | Pfliegler et al., 2014 |
|               |        |                     | Enology - botrytized      |          |             |                        |
| C. zemplinina | 11-124 | Hungary, Tarcal     | grape, 2003               |          | Debrecen    | Pfliegler et al., 2014 |
|               |        |                     | Enology - botrytized      |          |             |                        |
| C. zemplinina | 11-128 | Hungary, Tarcal     | grape, 2003               |          | Debrecen    | Pfliegler et al., 2014 |
|               |        | Hungary,            |                           |          |             |                        |
| C. zemplinina | 11-145 | Erdobénye           | Enology - botrytized must | Winery 2 | Debrecen    |                        |
| C. zemplinina | 11-149 | Hungary, Tarcal     | Enology - wine            | Winery 3 | Debrecen    | Pfliegler et al., 2014 |
| C. zemplinina | 11-150 | Hungary, Tarcal     | Enology - wine            | Winery 3 | Debrecen    | Pfliegler et al., 2014 |
|               |        | Switzerland,        |                           |          |             |                        |
| C. zemplinina | 11-18  | Waedenswill         | Enology - fermenting wine | Winery 4 | Debrecen    | Pfliegler et al., 2014 |
| C. zemplinina | 11-19  | Switzerland,        | Enology - fermenting wine | Winery 4 | Debrecen    | Pfliegler et al., 2014 |

|               |           | Waedenswill                             |   |                        |          |                        |
|---------------|-----------|---|---|------------------------|----------|------------------------|
|               |           | Switzerland,                            |   |                        |          |                        |
| C. zemplinina | 11-20     | Waedenswill                             | Enology - fermenting wine                       | Winery 4               | Debrecen | Pfliegler et al., 2014 |
|               |           |   | Enology - grape treated                         |                        |          |                        |
| C. zemplinina | 11-4      | Slovakia                                | With Kaptan                                     |                        | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-479    | Philippines, Manila                     | banana)   |                        | Debrecen | Pfliegler et al., 2014 |
| C zemplinina  | 11_6      | Italy Verona                            | Epology - fermenting must                       |                        | Debrecen | Pfliegler et al. 2014  |
| C. Zempinnia  | 11-0      | italy, verona                           | Wild - fly (Drosophila                          |                        | Deprecen | Fillegiel et al., 2014 |
| C. zemplinina | 11-60     | USA                                     | pinicola)                                       |                        | Debrecen | Pfliegler et al., 2014 |
| C zemplinina  | 11-9      | South Africa                            | Wild - soil                                     |                        | Debrecen | Pfliegler et al 2014   |
| e. zempinnu   | 11.5      | Italy, Castelluccio                     | Enology - fermenting must                       |                        | Debreeen |                        |
| C. zemplinina | 13C       | dei Sauri                               | (Uva di Troia), 2011                            |                        | Foggia   |                        |
|               |           |   | Enology - fermenting must                       |                        |          |                        |
| C. zemplinina | 1C        | Italy, Lucera                           | (Uva di Troia), 2011                            |                        | Foggia   |                        |
| C zomplining  | 20        | Italy Parlatta                          | Enology - fermenting must                       |                        | Foggia   |                        |
| C. zempiinina | 20        | italy, balletta                         | Fnology - fermenting must                       |                        | годдіа   |                        |
| C. zemplinina | 6C        | Italy, San Severo                       | (Uva di Troia), 2011                            |                        | Foggia   |                        |
|               |           |   | Enology - fermenting must                       |                        |          |                        |
| C. zemplinina | 7C        | Italy, Barletta                         | (Uva di Troia), 2011                            |                        | Foggia   |                        |
| <b>C</b>      | DA4 7     | <b>FD</b>                               | Enology - grape must                            |                        |          |                        |
| C. zemplinina | BAI-7     | France, Bourgogne                       | (Pinot noir), 2010                              |                        | 1000     |                        |
| C. zemplinina | BBM4VFA1  | France, Bourgogne                       | (Chardonnay), 2010                              | Winery 5               | IUVV     |                        |
|               |           |   | Enology - grape must                            |                        |          |                        |
| C. zemplinina | BBMV5FA17 | France, Bourgogne                       | (Chardonnay), 2010                              | Winery 5               | IUVV     |                        |
|               |           |   | Enology - grape must                            |                        |          |                        |
| C. zemplinina | BBMV6-3   | France, Bourgogne                       | (Chardonnay), 2010                              | Winery 5               | IUVV     |                        |
| C zemplinina  | BBS1EA3   | France Bourgogne                        | Enology - grape must                            | Winery 5               |          |                        |
| C. Zempinnia  | BBSILAS   | Trance, bourgogne                       | Enology - grape must                            | willery 5              | 10 V V   |                        |
| C. zemplinina | BBS2FA17  | France, Bourgogne                       | (Chardonnay), 2010                              | Winery 5               | IUVV     |                        |
|               |           | Italy, Friuli–Venezia                   | Enology - dried grapes                          |                        |          |                        |
| C. zemplinina | BC60      | Giulia                                  | must (Picolit)                                  |                        | DISAFA   | Urso et al., 2008      |
| C             | DT2C11    | France Deverses                         | Enology - fermenting must                       | Winery 6               | 1111.0.7 |                        |
| C. zemplinina | BISCII    | France, Bourgogne                       | (Chardonnay), 2011<br>Enclogy - fermenting must | (Sample 1)<br>Winery 6 | 10 V V   |                        |
| C. zemplinina | BT3C16    | France. Bourgogne                       | (Chardonnav), 2011                              | (Sample 1)             | Ιυνν     |                        |
| •             |           | , | Enology - fermenting must                       | Winery 6               |          |                        |
| C. zemplinina | BT3C18    | France, Bourgogne                       | (Chardonnay), 2011                              | (Sample 1)             | IUVV     |                        |
| <b>.</b>      | DTOOC     |   | Enology - fermenting must                       | Winery 6               |          |                        |
| C. zemplinina | BI3C6     | France, Bourgogne                       | (Chardonnay), 2011                              | (Sample 1)             | 1000     |                        |
| C. zemplinina | BTOC39    | France, Bourgogne                       | (Chardonnay), 2011                              | (Sample 2)             | IUVV     |                        |
|               |           |   | Enology - grape must                            | Winery 6               |          |                        |
| C. zemplinina | BTOC40    | France, Bourgogne                       | (Chardonnay), 2011                              | (Sample 2)             | IUVV     |                        |
|               |           |   | Enology - grape must                            | Winery 6               |          |                        |
| C. zemplinina | BIONSC44  | France, Bourgogne                       | (Chardonnay), 2011                              | (Sample 2)             | IUVV     |                        |
| C zemplinina  | BTONSC49  | France Bourgogne                        | (Chardonnay) 2011                               | (Sample 2)             |          |                        |
| 3. zempinnu   | 2.0.10010 |   | Enology - grape must                            | Winery 6               |          |                        |
| C. zemplinina | BTONSC50  | France, Bourgogne                       | (Chardonnay), 2011                              | (Sample 2)             | IUVV     |                        |
|               |           |   | Enology - grape must                            | Winery 6               |          |                        |
| C. zemplinina | BTONSC52  | France, Bourgogne                       | (Chardonnay), 2011                              | (Sample 2)             | IUVV     |                        |
| C zemplinina  | BTONSC56  | France Bourgogne                        | Chardonnay) 2011                                | (Sample 2)             |          |                        |
| C. Zempinnid  | 510105030 |   | Enology - fermenting                            |                        | 10 0 0   |                        |
|               |           |   | sweet botrytized musts,                         |                        |          |                        |
| C. zemplinina | CBS 9494  | Hungary, Tolcsva                        | 2001  | Winery 1               | CBS      |                        |

|               | 0704     |   | Enology - fermenting must | Vineyard 1 | DIGAGA  |  |
|---------------|----------|---|---------------------------|------------|---------|--|
| C. zemplinina | CZ01     | Italy, Asti wine area                   | (Barbera)                 | (Sample 3) | DISAFA  |  |
| C zomplining  | C702     | Italy Activing area                     | Enology - fermenting must | Vineyard 1 |         |  |
| C. Zempinnia  | 0202     | italy, Asti wille alea                  | Enclogy - fermenting must | Vinevard 1 | DISAFA  |  |
| C zemplinina  | C703     | Italy Asti wine area                    | (Barbera)                 | (Sample 3) | DISAFA  |  |
| 0.2011        | 0200     |   | Enology - fermenting must | Vinevard 2 | 2.0.1.1 |  |
| C. zemplinina | CZ04     | Italy, Asti wine area                   | (Barbera)                 | (Sample 4) | DISAFA  |  |
| · ·           |          |   | Enology - fermenting must | Vineyard 2 |         |  |
| C. zemplinina | CZ05     | Italy, Asti wine area                   | (Barbera)                 | (Sample 4) | DISAFA  |  |
|               |          |   | Enology - fermenting must | Vineyard 2 |         |  |
| C. zemplinina | CZ06     | Italy, Asti wine area                   | (Barbera)                 | (Sample 4) | DISAFA  |  |
|               |          |   | Enology - fermenting must | Vineyard 3 |         |  |
| C. zemplinina | CZ07     | Italy, Asti wine area                   | (Barbera)                 | (Sample 5) | DISAFA  |  |
|               |          |   | Enology - fermenting must | Vineyard 3 |         |  |
| C. zemplinina | CZ08     | Italy, Asti wine area                   | (Barbera)                 | (Sample 5) | DISAFA  |  |
| C zamalinina  | 6700     | Italy Activing area                     | Enology - fermenting must | Vincuard 4 |         |  |
| C. zemplinina | C209     | italy, Asti wine area                   | (Barbera)                 | Winory 6   | DISAFA  |  |
| C zomplining  |          | Franco Bourgogno                        | (Chardonnay) 2011         | (Sample 6) |         |  |
| C. Zempinnia  | DISNSI   | France, bourgogne                       | Enology - grane must      | Winery 6   | 10 V V  |  |
| C. zemplinina | DT3NS11  | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               | 2.0.021  |   | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS12  | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | Ιυνν    |  |
| · ·           |          | , | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS13  | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               |          |   | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS14  | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               |          |   | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS16  | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               |          |   | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS17  | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               | DT2NG40  | <b>FD</b>                               | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DI3NS18  | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | 1000    |  |
| C zemplinina  |          | France Bourgogne                        | (Chardonnay) 2011         | (Sample 6) |         |  |
| C. Zempinina  | D13N32   | France, bourgogne                       | Enclogy - grane must      | Winery 6   | 10 V V  |  |
| C zemplinina  |          | France Bourgogne                        | (Chardonnay) 2011         | (Sample 6) |         |  |
| C. Zempinina  | DISNOS   |   | Enology - grape must      | Winery 6   | 1011    |  |
| C. zemplinina | DT3NS4   | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | Ιυνν    |  |
| · ·           |          | , | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS5   | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               |          |   | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS6   | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               |          |   | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DT3NS7   | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
|               | 5-0100   |   | Enology - grape must      | Winery 6   |         |  |
| C. zemplinina | DI3NS8   | France, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | IUVV    |  |
| C zomplining  |          | France Pourgogne                        | Enology - grape must      | Winery 6   |         |  |
| C. Zempinina  | 0131039  | Grance, Bourgogne                       | (Chardonnay), 2011        | (Sample 6) | 10 V V  |  |
|               |          | Peloponnesus                            | Enology - fermenting must |            |         |  |
| C. zemplinina | E21NL17  | Nemea                                   | (Agiorgitiko)             | Vinevard 5 | DEMETER |  |
|               |          |   | Enology - fermenting must | Vineyard 6 |         |  |
| C. zemplinina | E222PL2  | Greece, Crete, Peza                     | (Kotsifali)               | (Sample 7) | DEMETER |  |
|               |          |   | Enology - fermenting must | Vineyard 6 |         |  |
| C. zemplinina | E222PL5  | Greece, Crete, Peza                     | (Kotsifali)               | (Sample 7) | DEMETER |  |
|               |          | Greece,                                 |                           |            |         |  |
|               |          | Peloponnesus,                           | Enology - fermenting must | Vineyard 7 |         |  |
| C. zemplinina | E228NL16 | Nemea                                   | (Agiorgitiko)             | (Sample 8) | DEMETER |  |
|               | 500000   | Greece,                                 | Enology - fermenting must | Vineyard 7 | DEN     |  |
| C. zemplinina | E228NL8  | Peloponnesus,                           | (Agiorgitiko)             | (Sample 8) | DEMETER |  |

|               |           | Nemea               |                           |              |           |                 |
|---------------|-----------|---------------------|---------------------------|--------------|-----------|-----------------|
|               |           |                     | Enology - fermenting must |              |           |                 |
| C. zemplinina | E244PL8   | Greece, Crete, Peza | (Kotsifali)               | Vineyard 8   | DEMETER   |                 |
| C zomplining  | E245DI 51 | Graaca Crota Baza   | Enology - fermenting must | Vinovard 8   | DEMETED   |                 |
| C. zempiinina | LZ4JFLJI  | Greece, Crete, Peza |                           | Villeyaru 8  | DLIVILILK |                 |
|               |           | Peloponnesus,       | Enology - fermenting must |              |           |                 |
| C. zemplinina | E27NL2    | Nemea               | (Agiorgitiko)             | Vineyard 9   | DEMETER   |                 |
|               |           | Greece,             |                           |              |           |                 |
|               |           | Peloponnesus,       | Enology - fermenting must |              |           |                 |
| C. zemplinina | E2NL510   | Nemea               | (Agiorgitiko)             | Vineyard 10  | DEMETER   |                 |
|               |           | Pelononnesus        | Enclosy - fermenting must |              |           |                 |
| C. zemplinina | E312NL11  | Nemea               | (Agiorgitiko)             | Vinevard 11  | DEMETER   |                 |
|               |           | Greece,             |                           | ,            |           |                 |
|               |           | Peloponnesus,       | Enology - fermenting must |              |           |                 |
| C. zemplinina | E326NL7   | Nemea               | (Agiorgitiko)             | Vineyard 9   | DEMETER   |                 |
|               |           |                     | Enology - fermenting must |              |           |                 |
| C. zemplinina | E348PL7   | Greece, Crete, Peza | (Mavroliatis)             | Vineyard 6   | DEMETER   |                 |
| C zemplinina  | F35DI 2   | Greece Crete Pezz   | Enology - termenting must | Vinevard 12  | DEMETER   |                 |
| C. Zempinnia  | LJJFLZ    | Greece, crece, reza | Enclosy - fermenting must | Villeyalu 12 | DEMETER   |                 |
| C. zemplinina | E427PL20  | Greece, Crete, Peza | (Vilana)                  | Vinevard 13  | DEMETER   |                 |
|               |           |                     | Enology - fermenting must |              |           |                 |
| C. zemplinina | E437PL9   | Greece, Crete, Peza | (Mavroliatis)             | Vineyard 14  | DEMETER   |                 |
|               |           |                     | Enology - fermenting must |              |           |                 |
| C. zemplinina | E438PL20  | Greece, Crete, Peza | (Mandilaria)              | Vineyard 14  | DEMETER   |                 |
| C             |           | Create Create Dese  | Enology - fermenting must | Via avand 0  | DEMAETER  |                 |
| C. zemplinina | E43PLI    | Greece, Crete, Peza | (KOTSITAII)               | vineyard 8   | DEIVIETER |                 |
| C zemplinina  | F510PL2   | Greece Crete Peza   | (Vilana)                  | Vinevard 15  | DEMETER   |                 |
| c. zempinina  |           |                     | Enology - fermenting must | Vineyard 16  | DEMETER   |                 |
| C. zemplinina | E52PL2    | Greece, Crete, Peza | (Vilana)                  | (Sample 9)   | DEMETER   |                 |
|               |           |                     | Enology - fermenting must | Vineyard 16  |           |                 |
| C. zemplinina | E52PL3    | Greece, Crete, Peza | (Vilana)                  | (Sample 9)   | DEMETER   |                 |
|               |           |                     | Enology - fermenting must |              |           |                 |
| C. zemplinina | E6PL30D   | Greece, Crete, Peza | (Kotsifall)               | Vineyard 12  | DEIVIETER |                 |
| C zemplinina  | FC54      | Giulia              | must (Picolit)            |              | DISAFA    | Urso et al 2008 |
| c. zempinina  | 1031      |                     | Enology - grape must,     |              |           |                 |
| C. zemplinina | L0311     | France, Sauternes   | 2003                      | Winery 7     | CRB Oeno  |                 |
|               |           |                     | Enology - grape must      |              |           |                 |
| C. zemplinina | L0471     | France, Mérignac    | (Merlot), 2004            | Winery 8     | CRB Oeno  |                 |
|               | 10470     |                     | Enology - grape must      |              | 000 0     |                 |
| C. zemplinina | L0472     | France, Merignac    | (IVIERIOT), 2004          | winery 8     | CKB Oeno  |                 |
| C. zemplinina | 10473     | France, Mérignac    | (Merlot), 2004            | Winery 8     | CRB Oeno  |                 |
|               |           |                     | Enology - grape must      |              |           |                 |
| C. zemplinina | L0629     | France, Mérignac    | (Merlot), 2006            | Winery 8     | CRB Oeno  |                 |
|               |           |                     | Enology - grape must      |              |           |                 |
| C. zemplinina | L0650     | France, Mérignac    | (Merlot), 2006            | Winery 8     | CRB Oeno  |                 |
|               |           |                     | Enology - grape must      |              |           |                 |
| C. zemplinina | LU651     | France, Merignac    | (IVIERIOT), 2006          | winery 8     | CKB Oeno  |                 |
| C. zemplinina | 10653     | France, Mérignac    | (Merlot), 2006            | Winery 8     | CRB Oeno  |                 |
| e. zempinnia  | 20000     |                     | Enology - fermenting must | thinking 0   |           |                 |
| C. zemplinina | L0656     | France, Mérignac    | (Merlot), 2006            | Winery 8     | CRB Oeno  |                 |
|               |           |                     | Enology - grape must      |              |           |                 |
| C. zemplinina | L0670     | France, Mérignac    | (Merlot), 2006            | Winery 8     | CRB Oeno  |                 |
|               | 10710     |                     | Enology - grape must      | 14/2 5       | 005.0     |                 |
| C. zemplinina | L0740     | France, Mérignac    | (Merlot), 2007            | Winery 8     | CRB Oeno  |                 |

|               |           | France, Saint-<br>Christophe-des- | Enology - grape must  |   |          |  |
|---------------|-----------|-----------------------------------|---|---|----------|--|
| C. zemplinina | L1405     | Bardes                            | (Merlot), 2013  | Winery 9                                    | CRB Oeno |  |
| C. zemplinina | L14105    | France, Barsac                    | Enology - high sugar grape<br>must (Semillon), 2013 Winery 10 |   | CRB Oeno |  |
| C. zemplinina | L14117    | France, Barsac                    | Enology - high sugar grape<br>must (Sauvignon), 2013          | high sugar grape<br>vignon), 2013 Winery 10 |          |  |
| C. zemplinina | L14123    | France, Sauternes                 | Enology -   | Winery 11                                   | ISVV     |  |
|               |           |                                   | Enology - high sugar grape                                    |   |          |  |
| C             | 114122    | France Contorney                  | must (Semillon and  | M/in a m. 12                                |          |  |
| C. zemplinina | L14132    | France, Sauternes                 | Muscatelle), 2013   | Winery 12                                   | CKB Oeno |  |
| C. zemplinina | L14151    | France, Sauternes                 | must, 2013  | Winery 7                                    | CRB Oeno |  |
| C. zemplinina | L1429     | France, Lussac                    | (Merlot), 2013  | Winery 13                                   | CRB Oeno |  |
| C. zemplinina | L1457     | France, Sauternes                 | Enology - high sugar grape<br>must (Sauvignon), 2013          | Winery 11                                   | CRB Oeno |  |
| C. zemplinina | 11464     | France, Sauternes                 | Enology -   | Winery 14                                   | ISVV     |  |
|               |           |                                   | Enology - high sugar grape                                    |   |          |  |
| C. zemplinina | L1479     | France, Sauternes                 | must (Semillon), 2013   | Winery 7                                    | CRB Oeno |  |
| C. zemplinina | L1485     | France, Sauternes                 | Enology - high sugar grape<br>must (Sauvignon), 2013          | Winery 7                                    | CRB Oeno |  |
|               |           |                                   | Enology - grape must  |   |          |  |
| C. zemplinina | MCR9      | France, Bourgogne                 | (Pinot noir), 2010  |   | IUVV     |  |
| C zomplining  | N711      | New Zealand,                      | Enology - fermenting must                                     | Winery 15                                   | CDDD     |  |
| C. Zempinina  | INZII     | New Zealand                       | Enclosy - fermenting must                                     | (Sample 10)<br>Winery 15                    | CRPR     |  |
| C. zemplinina | NZ12      | Napier                            | (Chardonnay), 2009  | (Sample 10)                                 | CRPR     |  |
|               |           | New Zealand,                      | Enology - fermenting must                                     | Winery 15                                   |          |  |
| C. zemplinina | NZ2       | Napier                            | (Chardonnay), 2009  | (Sample 10)                                 | CRPR     |  |
| C zamalinina  | NZC       | New Zealand,                      | Enology - fermenting must                                     | Winery 15                                   | CDDD     |  |
| C. Zemplinina | INZO      | New Zealand                       | Enclosy - fermenting must                                     | (Sample 10)<br>Winery 15                    | CKPK     |  |
| C. zemplinina | NZ8       | Napier                            | (Chardonnay), 2009  | (Sample 10)                                 | CRPR     |  |
|               |           |                                   | Enology - high sugar grape                                    |   |          |  |
| C. zemplinina | PE 102    | France, Barsac                    | must  | Winery 10                                   | ISVV     |  |
| C zemplinina  | DE 153    | France, Villenave                 | Enology - grape must  | Vinevard 17                                 | 15\/\/   |  |
| C. Zempinina  | 12155     | France, Villenave                 | Enology - grape must  | Villeyard 17                                | 1500     |  |
| C. zemplinina | PE 159    | d'ornon                           | (Merlot), 2012  | Vineyard 17                                 | ISVV     |  |
|               |           | France, Villenave                 | Enology - grape must  |   |          |  |
| C. zemplinina | PE 215    | d'ornon                           | (Merlot), 2012  | Vineyard 17                                 | ISVV     |  |
| C. zemplinina | PE 261    | France, Sauternes                 | Enology - nigh sugar grape<br>must                            | Winery 11                                   | ISVV     |  |
|               |           | ,                                 | Enology - high sugar grape                                    |   |          |  |
| C. zemplinina | PE 265    | France, Sauternes                 | must  | Winery 11                                   | ISVV     |  |
| C zemplinina  | PE 269    | France Sauternes                  | Enology - high sugar grape                                    | Winery 11                                   | 15\/\/   |  |
| C. Zempinina  | 1 2 2 0 5 | Trance, Sauternes                 | Enology - high sugar grape                                    | Which y 11                                  | 1500     |  |
| C. zemplinina | PE 272    | France, Sauternes                 | must  | Winery 12                                   | ISVV     |  |
|               |           |                                   | Enology - high sugar grape                                    | Winery 12                                   |          |  |
| C. zemplinina | PE 276    | France, Sauternes                 | must  | (Sample 11)                                 | ISVV     |  |
| C. zemplinina | PE 278    | France, Sauternes                 | must  | (Sample 11)                                 | ISVV     |  |
| C zomaliniza  | DE 270    |                                   | Enology - high sugar grape                                    | Winery 12                                   |          |  |
| C. Zemplinina | PE 2/9    | France, Sauternes                 | Fnology - high sugar grape                                    | (Sample 12)<br>Winery 12                    | 124.4    |  |
| C. zemplinina | PE 281    | France, Sauternes                 | must  | (Sample 12)                                 | ISVV     |  |
|               |           |                                   | Enology - high sugar grape                                    | Winery 12                                   | T        |  |
| C. zemplinina | PE 282    | France, Sauternes                 | must  | (Sample 12)                                 | ISVV     |  |
| C. zemplinina | PE 303    | France, Razac de                  | Enology - grape must  | Winery 16                                   | ISVV     |  |

|               |            | Saussignac            | (Merlot)                   |                           |        |  |
|---------------|------------|-----------------------|----------------------------|---------------------------|--------|--|
|               |            |                       | Enology - high sugar grape |                           |        |  |
| C. zemplinina | PE 387     | France, Sauternes     | must                       | Winery 14                 | ISVV   |  |
|               |            |                       | Enology - grape must       | Vineyard 18               |        |  |
| C. zemplinina | PE 399     | France, Ladaux        | (Merlot), 2012             | (Sample 13)               | ISVV   |  |
|               |            |                       | Enology - grape must       | Vineyard 18               |        |  |
| C. zemplinina | PE 400     | France, Ladaux        | (Merlot), 2012             | (Sample 13)               | ISVV   |  |
|               |            |                       | Enology - grape must       | Vineyard 18               |        |  |
| C. zemplinina | PE 401     | France, Ladaux        | (Merlot), 2012             | (Sample 13)               | ISVV   |  |
|               |            |                       | Enology - grape must       | Vineyard 19               |        |  |
| C. zemplinina | PE 455     | France, Cadaujac      | (Merlot), 2012             | (Sample 14)               | ISVV   |  |
|               |            |                       | Enology - grape must       | Vineyard 19               |        |  |
| C. zemplinina | PE 458     | France, Cadaujac      | (Merlot), 2012             | (Sample 14)               | ISVV   |  |
| C             | DE 460     | France Duisservin     | Enology - grape must       | Vineyard 20               |        |  |
| C. zemplinina | PE 460     | France, Puisseguin    | (Meriot), 2012             | (Sample 15)               | ISVV   |  |
| C zomalining  |            | France Duissaguin     | (Marlet) 2012              | Vineyard 20               |        |  |
| C. Zemplinina | PE 401     | France, Puisseguin    | (Meriot), 2012             | (Sample 15)               | 15 V V |  |
| C zemplinina  | DE 10      | France Barsac         | must                       | Winery 10                 | 15\/\/ |  |
| C. Zempinina  | FL 45      |                       | Enclogy - grane must       | Viney 10                  | 13 V V |  |
| C zemplinina  | PF 494     | France Ladaux         | (Merlot) 2012              | (Sample 16)               | ISVV   |  |
| C. Zempinnia  | 1 2 434    |                       | Enology - grane must       | Vinevard 18               | 13 V V |  |
| C zemplinina  | PF 495     | France Ladaux         | (Merlot) 2012              | (Sample 16)               | ISVV   |  |
| C. Zempinnu   | 12133      |                       | Enology - high sugar grape | (oumpic 10)               | 1011   |  |
| C. zemplinina | PF 89      | France, Sauternes     | must                       | Winery 11                 | ISVV   |  |
|               | . 2 00     |                       | Enology - high sugar grape |                           |        |  |
| C. zemplinina | PE 97      | France. Sauternes     | must                       | Winery 11                 | ISVV   |  |
|               |            | Italy, Friuli–Venezia | Enology - dried grapes     |                           |        |  |
| C. zemplinina | R5         | Giulia                | (Ramandolo)                |                           | DISAFA |  |
|               |            |                       | Enology - grape must       | Winery 17                 |        |  |
| C. zemplinina | Spain1     | Spain, Poboleda       | (Garnacha), 2012           | (Sample 17)               | URV    |  |
|               |            |                       | Enology - fermenting must  | Winery 18                 |        |  |
| C. zemplinina | Spain10    | Spain, Porrera        | (Garnacha), 2012           | (Sample 18)               | URV    |  |
|               |            |                       | Enology - fermenting must  | Winery 18                 |        |  |
| C. zemplinina | Spain11    | Spain, Porrera        | (Garnacha), 2012           | (Sample 18)               | URV    |  |
|               |            |                       | Enology - fermenting must  | Winery 18                 |        |  |
| C. zemplinina | Spain12    | Spain, Porrera        | (Garnacha), 2012           | (Sample 19)               | URV    |  |
|               |            |                       | Enology - fermenting must  | Winery 18                 |        |  |
| C. zemplinina | Spain13    | Spain, Porrera        | (Garnacha), 2012           | (Sample 19)               | URV    |  |
|               |            |                       | Enology - fermenting must  | Winery 18                 |        |  |
| C. zemplinina | Spain14    | Spain, Porrera        | (Garnacha), 2012           | (Sample 19)               | URV    |  |
| C             | Curain 1 F | Crain Constanti       | Enology - grape must       | Winery 19                 |        |  |
| C. zemplinina | Spain15    | Spain, Constanti      | (Xarel.io), 2013           | (Sample 20)               | URV    |  |
| C zomplining  | Spain?     | Spain Poholoda        | (Garnacha) 2012            | (Sample 17)               |        |  |
| C. Zempinina  | Spairiz    | Spain, Poboleua       | Enclogy - grane must       | (Janiple 17)<br>Wineny 17 |        |  |
| C zemplinina  | Spain3     | Snain Poholeda        | (Garnacha) 2012            | (Sample 17)               |        |  |
| C. Zempinnia  | Spanis     |                       | Enology - grane must       | Winery 17                 | 0111   |  |
| C. zemplinina | Spain4     | Spain, Poboleda       | (Garnacha), 2012           | (Sample 17)               | URV    |  |
|               | opanii     | opulli) i obolouu     | Enology - grape must       | Winery 17                 | 0      |  |
| C. zemplinina | Spain6     | Spain, Poboleda       | (Garnacha), 2012           | (Sample 17)               | URV    |  |
|               |            | Spain, Morera del     | Enology - fermenting must  | ,,                        |        |  |
| C. zemplinina | Spain7     | Montsant              | (Cariñena), 2012           | Winery 20                 | URV    |  |
|               |            |                       | Enology - fermenting must  |                           |        |  |
| C. zemplinina | Spain8     | Spain, Escaladei      | (Cariñena), 2012           | Winery 21                 | URV    |  |
|               |            |                       | Enology - fermenting must  | Winery 18                 |        |  |
| C. zemplinina | Spain9     | Spain, Porrera        | (Garnacha), 2012           | (Sample 19)               | URV    |  |
|               | UWOPS 07-  |                       | Wild - fruit (Osage        |                           |        |  |
| C. zemplinina | 402.2      | Canada, London        | Orange)                    |                           | UWOPS  |  |
|               | UWOPS 83-  |                       | Wild - fruit (Opuntia      |                           |        |  |
| C. zemplinina | 775.2      | Bahamas               | stricta)                   |                           | UWOPS  |  |

|               | UWOPS 91- |             |                       |       |  |
|---------------|-----------|-------------|-----------------------|-------|--|
| C. zemplinina | 743.1     | USA, Hawaii | Wild - fly (Sapindus) | UWOPS |  |
| C. stellata   | CBS 157   | N/A         | Enology - wine        | CBS   |  |

#### 564 **Table 2. Microsatellite loci for** *Candida zemplinina* genotyping.

565 Allele size in pb. Forward primers were tailed on 5'-end with M13 sequence (CACGACGTTGTAAAACGAC). Tm is the melting temperature

566 used for microsatellite amplification (see Materials and Methods).

| Microsatellite<br>name | Motif           | Fluorescent<br>dye | Primers                   | Tm | Alleles size (repeats<br>number) for CBS<br>9494T | Alleles size<br>(repeats number)<br>range   |
|------------------------|-----------------|--------------------|---------------------------|----|---|---|
| C713                   |                 | FAM                | F: TTGCGAATGTGTTTCGGA;    | 55 | 125 (21)  | 101-125 (13-21)   |
| 0210                   | 10, () 100, 100 |                    | R: ATGAGAAGGCCGAGGACGAT   | 55 | 120 (21)  | ats       Alleles       size         BS       (repeats       number)         range       101-125 (13-21)         289-304 (18-23)       289-304 (18-23)         271-361 (9-54)       109-118 (8-11)         152-172 (7-17)       278-299 (6-13)         112-133 (14-21)       265-319 (7-25)         154-170 (15-23)       100-115-23) |
| C745                   | стт/сст         | PFT                | F: TCCAGCTCGGCAATATCAAT;  | 55 | 298 (21)  | 289-301 (18-23)   |
| 0245                   | 611/001         |                    | R: TGACGAGGAGAACAGTGAAGA  | 55 | 250 (21)  | 205 504 (10 25)   |
| C711                   |                 | ΕΔΝΛ               | F: TGCGATTATACTATTTTGCGA; | 55 | 339 (13)  | 271-361 (9-54)  |
| C211                   |                 |                    | R: TGCGAAAAGAACGACAGGAA   | 55 | 555 (45)  | 271-301 (3-34)  |
| C733                   |                 | нгу                | F: TGGCTATACCGATTTTGGTGA; | 55 | 115 (10)  | 109-118 (8-11)  |
| C233                   |                 |                    | R: TGTCCTAATTCCTCTCTCGTC  | 55 | 115 (10)  |   |
| C71                    | GT              | НЕХ                | F: AAGAACGTTGGTAGGCCTGAA; | 55 | 168 (15)  | 152-172 (7-17)  |
| CZ1                    | 01              |                    | R: GGGTTCAATTCAATGTTCGG   | 55 | 100 (15)  |   |
| C715                   | CAA             | нгх                | F: AACTTGCGCAACAAGTGTTGA; | 55 | 299 (13)  | 278-299 (6-13)  |
| C215                   | CAA             |                    | R: TGATTCTGCATTTGTCCTGG   | 55 | 235 (13)  | 278-233 (0-13)  |
| C720                   |                 |                    | F: ATACCTGGTAGCCCGAATGC;  | 52 | 130 (20)  | 112-133 (14-21)   |
| CZ20                   | ACA/ UCA        |                    | R: TTTGATTGTTGCTGTTGCTG   | 52 | 130 (20)  |   |
| C754                   |                 |                    | F: AAAATAAACCGGCTAGCGGTG; | 55 | 301 (10)  | 265-319 (7-25)  |
| CZJ4                   | AUA             |                    | R: TCCTTTCTCCATCCTGAGACA  | 55 | 501 (15)  | 205-515 (7-25)  |
| C750                   |                 | DET                | F: ATATAAACACCCACCGCCACA; | 55 | 170 (22)  | 15/ 170 (15 22)   |
| C233                   |                 | r <b>L</b> I       | R: TTGCAGATTGAGCATTGCAC   | 55 | 1/0 (23)  | 134-110 (13-23)   |
| C74                    | тст             | DET                | F: CCATATGCGCATCAACATCA;  | 55 | 248 (15)  | 226 251 (11 16)   |
| C24                    |                 |                    | R: ATGGTAGCTGACGCTACTGGT  | در | 240 (13)  | 230-231 (11-10)   |

#### 568 Figure legends

- 569 Figure 1. Genetic relationships between 163 *C. zemplinina* strains using ten 570 microsatellite markers.
- 571 A: Dendrogram tree built using Euclidean distance and Neighbor-Joining's clustering. The
- 572 robustness of the node was assessed using multiscale bootstrap resampling and approximated
- 573 unbiased test (n = 1000 boots).
- 574 B: Barplot representing STRUCTURE results (K = 4). The posterior probability (y-axis) of
- 575 assignment of each strain (vertical bar) to ancestral groups is shown by colors (green, yellow,
- 576 orange and pink represent each 4 ancestral populations).



- 578 Figure 2. Genetic relationships between isolates from the same vineyard/winery.
- 579 Strains isolated from 7 vineyards/wineries (in 7 different countries) were localised on the
- 580 dendrogram tree.

