Patricia Velez[a,*], Jaime Gasca-Pineda[b], Akira Nakagiri, Richard T. Hanlin and María C. González

Genetic diversity and population structure of *Corollospora maritima sensu lato*: new insights from population genetics

DOI 10.1515/bot-2016-0058
Received 22 June, 2016; accepted 24 August, 2016; online first 26 September, 2016

Abstract: The study of genetic variation in fungi has been poor since the development of the theoretical underpinnings of population genetics, specifically in marine taxa. *Corollospora maritima sensu lato* is an abundant cosmopolitan marine fungus, playing a crucial ecological role in the intertidal environment. We evaluated the extent and distribution of the genetic diversity in the nuclear ribosomal internal transcribed spacer region of 110 isolates of this ascomycete from 19 locations in the Gulf of Mexico, Caribbean Sea and Pacific Ocean. The diversity estimates demonstrated that *C. maritima sensu lato* possesses a high genetic diversity compared to other cosmopolitan fungi, with the highest levels of variability in the Caribbean Sea. Globally, we registered 28 haplotypes, out of which 11 were specific to the Caribbean Sea, implying these populations are genetically unique. We detected populations inhabiting human-impacted sites with null genetic variation. As long-term exposure to contaminants has been proven to decrease genetic diversity, a conservation genetics approach to assess this matter is urgent. Our results revealed the occurrence of five genetic lineages with distinctive environmental preferences and an overlapping geographical distribution, agreeing with previous studies reporting physiological races within this species.

Keywords: dispersal; gene flow; ITS rDNA; marine Ascomycota; molecular ecology.

[a]Present address: Departamento de Microbiología Experimental, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California Sur 22860, Mexico
[b]Present address: Departamento de Biología de la Conservación, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California Sur 22860, Mexico
***Corresponding author: Patricia Velez,** Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-233, Mexico City 04510, Mexico, e-mail: pvelezaguilar@gmail.com. http://orcid.org/0000-0002-4449-8977
Jaime Gasca-Pineda: Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, Mexico City 04510, Mexico
Akira Nakagiri: Division of Genetic Resource Preservation and Evaluation, Fungus/Mushroom Resource and Research Center, Tottori University, Tottori 6808553, Japan
Richard T. Hanlin: Mycology Herbarium, Museum of Natural History Annex, University of Georgia, 4435 Atlanta Highway, Athens, GA 30606, USA
María C. González: Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-233, Mexico City 04510, Mexico

Introduction

Sandy beach ecosystems harbor a unique biodiversity, which is highly adapted to endure dynamic and extreme conditions. This biodiversity performs critical habitat functions, providing a range of ecological services not available through other ecosystems (McLachlan and Brown 2006, Schlacher and Connolly 2009). However, the widespread modification of sandy beaches and further intense anthropogenic pressures (predicted to intensify over the next few decades) have led to an irreversible loss of biodiversity (Roberts and Hawkins 1999, Brown et al. 2008). The loss of functional capacities of this system could have irreparable damage on larger ecosystem scales (Schlacher et al. 2006, Defeo et al. 2009).

The limited scientific understanding of the genetic variation within populations is an impediment for the conservation and management of threatened ecosystems. Therefore, it has become increasingly critical to conduct studies assessing the genetic patterns of species occurring in the intertidal zone. Understanding the evolutionary and ecological significance of DNA variation within species represents an important task available through population genetics (Hedrick 2011). This discipline represents a useful tool to explore numerous areas of mycology dealing with the dynamics among populations in a space and time scale (Douhan et al. 2011). However, since the development of its theoretical underpinnings in the 1920s and 1930s, the study of genetic variation in non-model fungal populations has been poor compared to other organisms.

Recently, with the development of accessible molecular markers and novel cutting-edge technologies, our understanding of the diversity, evolution and ecology of marine fungi has been improved (e.g. Spatafora et al. 1998, Kohlmeyer et al. 2000, Abdel-Wahab et al. 2001, Inderbitzin et al. 2002, 2004, Stoeck and Epstein 2003, Campbell et al. 2005, Schoch et al. 2007, Zuccaro et al. 2008, Jones et al. 2009, Chalkley et al. 2010, Abdel-Wahab 2011, Pang et al. 2013, Rämä et al. 2014, Jones et al. 2015, Velez et al. 2015a, 2016). Nonetheless, literature on the population genetics of marine fungi is virtually lacking. Since the pioneer work on the worldwide distributed asexual species *Paradendryphiella arenaria* (Nicot) Woudenberg *et* Crous and *P. salina* (G.K. Sutherland) Woudenberg *et* Crous, only a preliminary report on the arenicolous species *Corollospora maritima* Werdermann has been added to the literature (Michaelis et al. 1987, Velez et al. 2016). Therefore, the extent, distribution and unique features of the genetic diversity in fungal species inhabiting marine environments are yet to be characterized, emphasizing surveys at a population level of widely distributed taxa.

The cosmopolitan ascomycete, *C. maritima* is the most abundant and well-studied member of arenicolous fungi (Kohlmeyer 1983, Pugh and Jones 1986, Volkmann-Kohlmeyer and Kohlmeyer 1993, González et al. 1998, Jones 2000, Figueira and Barata 2007). Remarkably, this ascomycete represents a valuable genetic resource from the anthropogenic point of view, as it is a candidate species for oil spill bioremediation, it has been proposed as a bioindicator of beach degradation by touristic activities, and produces isobenzofuranone-type compounds with antibacterial activity (Kirk and Gordon 1988, Kirk et al. 1991, Liberra et al. 1998, González and Hanlin 2010). Equally, this dominant fungus also plays a crucial ecological role in intertidal environments as a saprobe, decomposing a wide range of substrata (Kohlmeyer and Kohlmeyer 1979, Grant et al. 1996).

Based on the observation of differential growth rates in five isolates of *C. maritima* from different locations, it has been proposed that this species comprises several physiological races with different temperature requirements. However, races cannot be morphologically distinguished (Bebout et al. 1987). Similarly, the occurrence of geographical races has been suggested based on the PCR RAPD analysis of 10 isolates from temperate, subtropical and tropical regions (Roberts et al. 1995). Recently, a pioneer study based on inter-simple sequence repeats (ISSR) suggests the occurrence of several genetic groups (Velez et al. 2016). Nevertheless, no conclusive survey has been conducted. In the rest of this paper, therefore, we will refer to *C. maritima* in the broad sense, since cryptic speciation has been suspected (Kohlmeyer and Charles 1981, Bebout et al. 1987, Roberts et al. 1995, Pang et al. 2011).

Cosmopolitan taxa commonly comprise complexes of regionally more restricted forms, and in some cases of cryptic species. Biological examples in the marine environment include calcareous sponges, fish, and diatoms (Solé-Cava et al. 1991, Graves 1998, Casteleyn et al. 2008). In fungi, a large number of species have a broad geographical distribution. However, little is known about the genetic structure (dispersal abilities) and genetic variation of these species. Several studies on terrestrial taxa indicate the occurrence of cryptic taxa and strong geographical structure, proposing dispersal restrictions, recent population establishment, and potential endemism (James et al. 1999, Carriconde et al. 2008, Ngamskulrungroj et al. 2009). Nevertheless, low genetic variation and an absence of population structure have also been reported in cosmopolitan fungal species such as *Aspergillus fumigatus* (Rydholm et al. 2006).

Here we present the first analysis of the genetic diversity at a population level of 110 isolates of the cosmopolitan marine ascomycete *C. maritima sensu lato* from 19 sites in several littoral zones, focusing on Mexican material, using the internal transcribed spacer (ITS) region of the rDNA. We discuss the extent of genetic variation, and the genetic structure in relation to some relevant environmental variables and the geographic distribution of the species.

Materials and methods

Sampling and isolation

We sampled 19 marine sandy beaches for *Corollospora maritima sensu lato* on the coastlines of Japan, Cuba, the USA, Panama, Ecuador and Mexico (Table 1, Figure 1). In order to obtain the fungi, we followed the collecting and incubation techniques described by Kohlmeyer and Kohlmeyer (1979). Samples were taken from the mesobeach at each site (Carranza-Edwards and Caso-Chávez 1994). Wood pieces, algae and other debris were collected randomly, placed in a sterile hermetic plastic bag and covered with moist sand from the collecting sites. We incubated the samples for 6 months under laboratory conditions. At the end of the incubation period, samples were examined with a stereomicroscope Nikon SMZ1000 (Tokyo, Japan) and a compound microscope (Nikon Eclipse 80i) for the morphological identification of *C. maritima sensu lato* (Kohlmeyer and Kohlmeyer 1979, Kohlmeyer and Volkmann-Kohlmeyer 1991, Jones et al. 2009). Further taxonomical confirmation

Table 1: Location of the sampled beaches and information for each site, biogeographic regions are designated according to Bebout et al. (1987).

Site	Beach	Location	Region	Biogeographic province	Coordinates
CA	Cancún	Quintana Roo, Mexico	Caribbean Sea	Tropical	21°05′23.40″N, 86°46′06.04″W
CU	Bahía de Cochinos	Matanzas, Cuba		Tropical	22°16′48.33″N, 81°12′00.52″W
PR	Progreso	Yucatán, Mexico		Tropical	21°17′18.07″N, 89°39′50.88″W
DB	Dzilam de Bravo	Yucatán, Mexico		Tropical	21°23′26.96″N, 88°54′19.16″W
AC	Acapulco	Guerrero, Mexico	Eastern Pacific Ocean	Tropical	16°51′26.81″N, 99°52′12.49″W
PV	Puerto Vallarta	Jalisco, Mexico		Tropical	20°38′20.71″N, 105°14′11.55″W
PA	Farfán Beach	Panamá, Panama		Tropical	8°56′21.99″N, 79°33′55.96″W
EC	Salinas	Santa Elena, Ecuador		Tropical	2°11′52.41″S, 80°58′53.17″W
LP	La Paz	Baja California Sur, Mexico		Temperate	24°10′29.71″N, 110°18′17.38″W
SO	Puerto Peñasco	Sonora, Mexico		Temperate	31°19′26.22″N, 113°35′13.75″W
MI	Miramar	Tamaulipas, Mexico	Gulf of Mexico	Tropical	22°17′49.11″N, 97°48′24.97″W
ES	Escolleras	Tamaulipas, Mexico		Tropical	22°15′52.77″N, 97°47′04.81″W
TU	Tuxpan	Veracruz, Mexico		Tropical	20°58′22.32″N, 97°18′22.32″W
NA	Nautla	Veracruz, Mexico		Tropical	20°12′52.16″N, 96°45′32.34″W
BR	Boca del Rio	Veracruz, Mexico		Tropical	19°07′19.97″N, 96°06′17.11″W
TA	Paraíso	Tabasco, Mexico		Tropical	18°26′24.88″N, 93°13′33.34″W
CC	Ciudad del Carmen	Campeche, Mexico		Tropical	18°39′55.75″N, 91°48′38.09″W
HA	Kauai	Hawaii, USA	Western Pacific Ocean	Tropical	22°04′15.80″N, 59°46′28.92″W
DU	Sand Dunes	Tottori, Japan		Temperate	35°32′46.77″N, 134°13′49.35″W

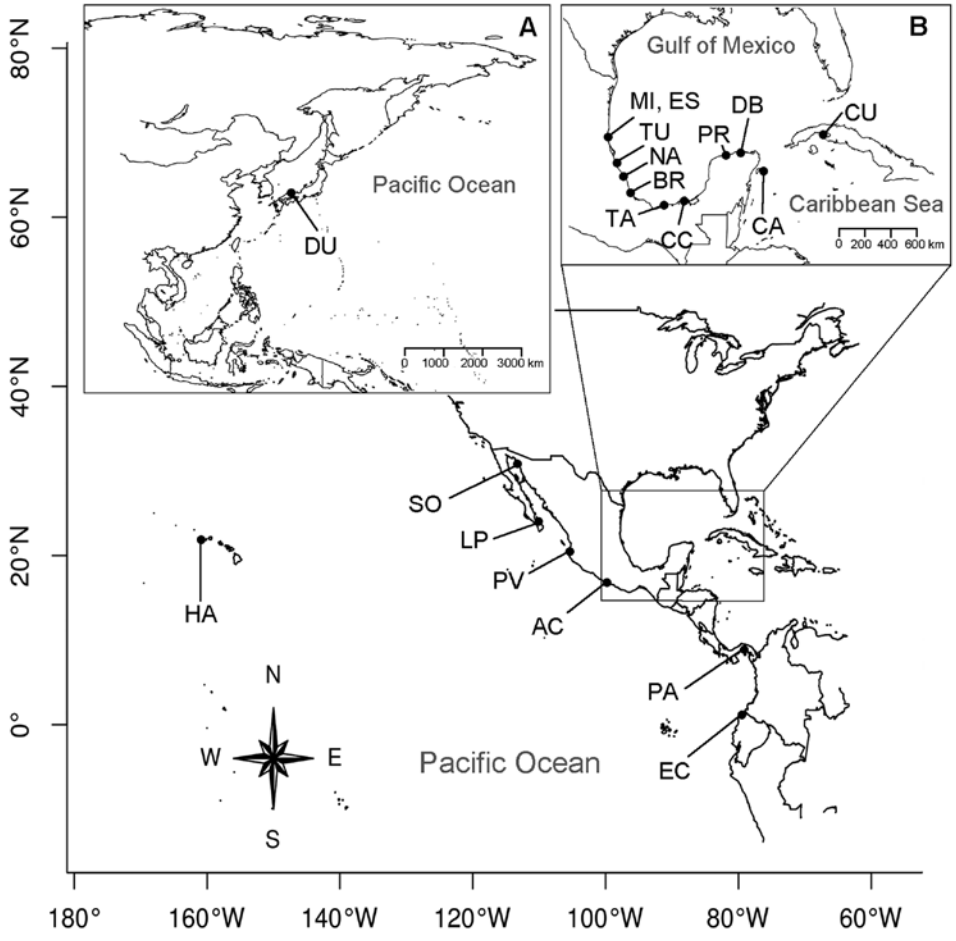

Figure 1: Geographical location of populations of *C. maritima sensu lato* studied. (A) Japan. (B) Gulf of Mexico and Caribbean Sea. Site nomenclature corresponds to list of localities in Table 1.

at a genus level was conducted based on the ITS sequences assessment as high intraspecific variation was expected.

All cultures used in this study were isolated *de novo* to avoid erroneous estimations of genetic diversity due to the accumulation of mutations or loss of genetic diversity resulting from repeated transfers of cultures over long periods of storage (e.g. Ratcliff et al. 2012). Only mature material exhibiting the standard morphology of the species as described by Werdermann (1922) and Kohlmeyer and Volkmann-Kohlmeyer (1991) was considered (Supplemental Table S1). We obtained one single-spore isolate per sample. Isolates were grown at room temperature in potato dextrose agar medium (Sigma-Aldrich, St. Louis, MO, USA) with added artificial seawater (Instant Ocean®, Aquarium Systems, USA; Choi et al. 1999). Dry specimens, isolates, as well as total DNA samples were deposited in the mycological collection of the Laboratory of Biology and Diversity of Marine and Freshwater Ascomycetes from Mexico, headed by Dr. María C. González, in the Instituto de Biología, Universidad Nacional Autónoma de México, and are available for research upon request.

DNA extraction, ITS amplification, and sequencing

Isolates were grown for 15 days at 25°C in 50 ml potato-dextrose liquid medium (Fluka Cat. # P6685, Sigma-Aldrich, St. Louis, MO, USA) supplemented with artificial seawater (Instant Ocean) following the instructions given by the manufacturer. Total genomic DNA was extracted using the cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle 1987). The DNA samples were stored at 4°C until used.

Aliquots (0.4 µM) of DNA samples were used as templates in a polymerase chain reaction (PCR). Amplification was carried out using the primer pair ITS1/ITS4 in a 50-µl reaction volume (White et al. 1990). Cycling parameters included an initial denaturation at 94°C for 3 min followed by 35 cycles at 94°C for 45 s, annealing at 55°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 10 min, after which the reaction was cooled to a constant 4°C. The PCR products were commercially sequenced in both directions (University of Washington High-Throughput Sequencing Center, USA).

Bioinformatics and statistical analyses

The quality examination and edition of the sequences were performed using Consed software version 27.0 (Ewing and Green 1998, Ewing et al. 1998, Gordon et al. 2001), and alignment was conducted with MUSLCLE version 3.8.31 (Edgar 2004). The GenBank Data Base accession numbers of *Corollospora maritima sensu lato* sequences are provided as Supplemental Table S2.

As alignments of fungal ITS sequences have multiple insertion-deletion regions (Nagy et al. 2012), nucleotide diversity (π), number of segregant sites (S), number of haplotypes (H), and Tajima's D were estimated excluding the gaps in paired comparisons in DnaSP v5.10.01 (Librado and Rozas 2009). To evaluate the genetic distances between localities, Nei's genetic distance was estimated (Nei 1978), and a distance dendrogram using Ward's clustering criterion was built (Murtagh and Legendre 2014), using the package "adegenet" (Jombart and Ahmed 2011), R version 3.2.0 (R Core Team 2015). For the analyses, the study sites were grouped into four regions based on oceanographic characteristics, such as superficial sea currents and geographical proximity (Table 1).

The assembled sequences were compared to the GenBank Data Base through a BLAST search in order to obtain reference sequences for the phylogenetic analyses. We preferably included accessions associated with culture collections, resulting in six sequences of *C. maritima* (JN943388/strain NBRC 32117, KM272367/strain 01092011III.3, JN943387/strain NBRC 32118, AB361029/strain MD 812, AB361028/strain MD 831, AB361027/strain MD 835), *C. gracilis* (JN943386/strain NBRC 32111), *C. cinnamomea* (AB361023/strain NBRC 32125) and *Halosigmoidea parvula* (FJ591159/strain NBRC 32159). A final alignment was conducted, including reference sequences from GenBank using MUSLCLE version 3.8.31 (Edgar 2004). The sequences were clustered using BLASTClust implemented in the MPI Bioinformatics Toolkit (Biegert et al. 2006), with a 94% similarity criterion as proposed by Millberg et al. (2015) for presumed genus level.

To explore the phylogenetic relationships among isolates, a Bayesian reconstruction using MrBayes version 3.2.2 (Ronquist et al. 2012) was performed with the following settings: four parallel runs with one cold chain and four heated chains each for 10,000,000 generations starting with a random tree and a sampling scheme every 1000 steps. We used *Lecanicillium kalimantanense* Kurihara *et* Sukarno and *C. angusta* Nakagiri *et* Tokura (NR_121200/strain BTCC F23 and KT207764/strain KUC21246, respectively) as outgroups. The substitution model used was HKY+G, obtained by jModel Test version 2.1.7 (Darriba et al. 2012); the model was selected considering both AIC and BIC criteria. The tree was visualized using FigTree version 1.4.2 (Rambaut 2009). Additionally, a minimum spanning tree (MST) among haplotypes was constructed

using the Tamura-Nei distance (Tamura and Nei 1993) in Arlequin version 3.5 (Excoffier and Lischer 2010), and the network was visualized using HapStar version 0.7 (Teacher and Griffiths 2011).

To assess habitat preferences (here defined as environmental variables), influencing the distribution of the genetic lineages (clusters obtained in the BLASTClust analysis), a constrained correspondence analysis (CCA) was performed (Ter Braak 1986, Legendre and Legendre 2012). We evaluated 35 environmental variables from Bioclim (Hijmans et al. 2005) and Climond (Kriticos et al. 2012) databases. To determine the set of non-redundant and explanatory variables, the number of variables was reduced by means of three statistical criteria. First, a Pearson-correlation matrix was conducted using the z-scores of the variables to identify the ones with correlation values >0.80, then a principal component analysis (PCA) was performed in order to assess the most explanatory variables by inspecting their loadings; these analyses were performed using the packages "vegan" version 2.3-1 (Oksanen et al. 2015), "ade4" version 1.7.2 (Dray and Dufour 2007), and "FactoMineR" (Husson et al. 2016) in R. Finally a colinearity diagnostic implemented by the function *colldiag* of the package "perturb" (Hendrickx 2012) was used to obtain a condition index >30, as suggested by Belsley et al. (1980). After the variable exclusion, 10 variables were retained: isothermality, temperature seasonality, mean temperature of wettest quarter, precipitation of driest quarter, radiation of wettest quarter, radiation of coldest quarter, moisture index seasonality, mean moisture index of warmest quarter, mean moisture index of coldest quarter, radiation of driest quarter.

Results

Genetic diversity

The alignment of the ITS sequences of *Corollospora maritima* isolates was 490 bp long. No significant differences were found in the 5.8S rDNA region, with all of the genetic variation occurring in the ITS1 and ITS2 regions (Figure 2). In the 110 analyzed isolates of *C. maritima sensu lato* from 19 locations in the Gulf of Mexico, Caribbean Sea and Pacific Ocean, a high overall genetic diversity ($h = 28$, $S = 150$, $\pi = 0.0882$) was detected. In terms of the geographical origin of the isolates, the highest genetic diversity was observed in the Caribbean Sea, whereas the Gulf of Mexico harbored the lowest levels. Moreover, at the beach level, the isolates from CU followed by DU showed the highest

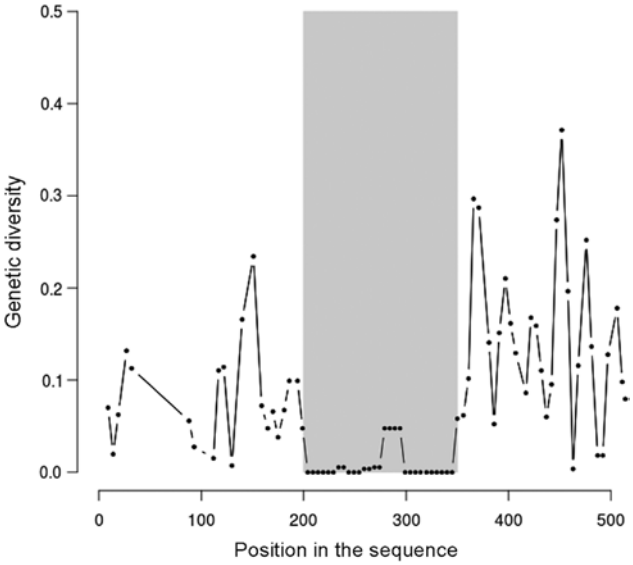

Figure 2: Genetic diversity within the nuclear ribosomal internal transcribed spacer region, including the 5.8S rDNA coding region (shaded area) in the sampled isolates of *Corollospora maritima sensu lato* from several sites in the Pacific Ocean, Caribbean Sea and Gulf of Mexico.

diversity, while isolates from BR, ES, NA, AC, LP, PV, PA, EC, HA possessed no genetic variation (Table 2). Also, differences in the D parameter were observed ranging from $D = 3.4594$ and 1.6208 in the Eastern Pacific Ocean and the Gulf of Mexico regions, respectively, to $D = 0.1134$ and 0.3902 in the Western Pacific Ocean and the Caribbean Sea regions, respectively.

Genetic differentiation and population structure

No particular geographical tendency was observed. Study site clustering based on Nei's genetic distance (number of substitutions per locus that have occurred after divergence of two populations), as well as Edwards' genetic distance (Euclidean representation of the distance between populations with differing gene frequencies) did not resemble the geographical distribution of populations, grouping locations from the Caribbean Sea, Gulf of Mexico and Western Pacific together (Figure 3A, B; Edwards 1971, Nei 1972).

The phylogenetic analysis distinguished five co-distributed genetic lineages in the sampled isolates (Figure 4), yet no geographical assemblages could be identified. The first lineage encompassed isolates from SO, LP (Eastern Pacific Ocean), CC, MI (Gulf of Mexico), DU (Western Pacific Ocean), DB, PR, and CU (Caribbean Sea); the second cluster included only isolates from CU

Table 2: Summary statistics of the genetic diversity within the 19 studied populations of *Corollospora maritima sensu lato* in the Pacific Ocean, Caribbean Sea and Gulf of Mexico.

Region	Locality	*N*	*H*	*S*	π	*D*
Caribbean Sea	CA	5	5	35	0.0297	
	DB	9	3	3	0.0030	
	PR	5	2	2	0.00154	
	CU	4	3	107	0.1286	
	Total	23	13	136	0.0872	0.3902 ($p > 0.10$)
Gulf of Mexico	BR	10	1	0	0	
	CC	9	3	96	0.0645	
	ES	6	1	0	0	
	MI	8	2	75	0.0395	
	NA	7	1	0	0	
	TA	7	3	2	0.0010	
	TU	8	2	1	0.00051	
	Total	55	9	98	0.0633	1.6208 ($p > 0.10$)
Eastern Pacific Ocean	AC	1	1	0	0	
	LP	4	1	0	0	
	PV	3	1	0	0	
	SO	6	2	1	0.0006	
	PA	2	1	0	0	
	EC	3	1	0	0	
	Total	19	6	77	0.0833	3.4594 ($p < 0.001$)
Western Pacific Ocean	HA	9	1	0	0	
	DU	4	2	78	0.0833	
	Total	13	3	116	0.0782	0.1134 ($p > 0.10$)
	Total sample	110	28	150	0.0882	1.5989 ($p > 0.10$)

N, Number of analyzed sequences; *H*, number of haplotypes; *S*, number of segregating sites; π, nucleotide diversity; *D*, *Tajima's D* statistical test for non-neutral evolution. For site nomenclature, see Table 1.

(Caribbean Sea); the third cluster contained isolates from HA (Western Pacific Ocean); the fourth comprised isolates from CC (Gulf of Mexico), and CA (Caribbean Sea); and the fifth included isolates from BR, CC, ES, MI, NA, TA, TU (Gulf of Mexico), CA, CU (Caribbean Sea), DU (Western Pacific Ocean) AC, EC, PA, and PV (Eastern Pacific Ocean). Interestingly, reference sequences MD835, MD831, MD817 (Egypt), 32117 (Japan) and 01092011 grouped within the first genetic lineage, whereas 32118 (Japan) lay within lineage five. Moreover, several private haplotypes at a lineage and littoral levels were identified. The dominant haplotype H9 from lineage 1 was recorded from populations in the Gulf of Mexico, Caribbean Sea and Eastern Pacific (Figure 4).

Environmental affinities

Through a multivariate constrained ordination technique, we were able to explore the relationships between genetic lineages and some environmental variables (Figure 5). Our results indicated that the five genetic lineages of *Corollospora maritima sensu lato* discovered in this study possess distinctive environmental preferences, with lineages 2 and 4 being the most similar. Our findings suggest that seasonal fluctuations in temperature, and solar radiation in the wet quarter of the year may have an important influence on the occurrence of lineage 1. Moreover, solar radiation during the cold quarter of the year has a strong effect on the distribution of genetic lineage 3, whereas mean moisture during the warm quarter of the year represents an important environmental determinant for the occurrence of genetic lineage 5 (Figure 5).

Discussion

Genetic diversity

The remarkably high levels of genetic variation in *Corollospora maritima sensu lato* ($\pi = 0.088$) are in contrast to those found in previous genetic studies on fungi, which were within moderate genetic diversity values: the grape powdery mildew fungus *Erysiphe necator* ($\pi = 0.00194$), the widely-distributed lichen species

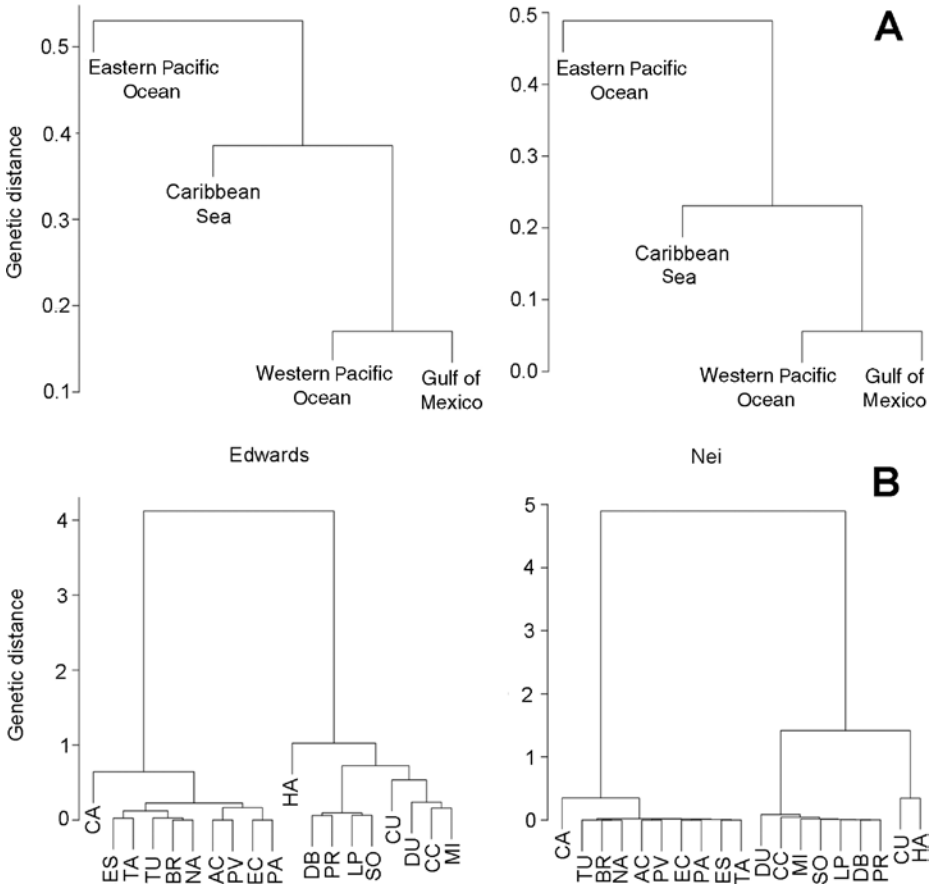

Figure 3: Clustering of worldwide *Corollospora maritima sensu lato* isolates from different geographical regions based on genetic distances among populations (A), and (B) among sites. Scale of y-axis represents the genetic distance. For site nomenclature, refer to Table 1.

Cetraria aculeata and *Xanthoria parietina* ($\pi = 0.0031$), and the saprobic Japanese endemic species *Dasyscyphella longistipitata* (0.0028), to higher diversity levels as in the parasitic species *Ophiocordyceps sinensis* ($\pi = 0.008$), and the cosmopolitan arbuscular mycorrhizal species *Glomus mosseae* ($\pi = 0.007$) (Lindblom and Ekman 2006, Rosendahl et al. 2009, Zhang et al. 2009, Brewer and Milgroom 2010, Hosoya et al. 2010, Domaschke et al. 2012). Moreover, our findings on the high genetic diversity within *C. maritima sensu lato* are also different from previous studies on worldwide distributed marine asexual species (*P. arenaria* and *P. salina*), where low diversity levels were reported (Michaelis et al. 1987). Yet, such estimates could have significantly underestimated genetic diversity since they were based on electromorph variation (Lewontin 1991).

Our results on the remarkably high genetic variability of *C. maritima sensu lato* agree with described genetic patterns for other marine organisms, which generally possess higher genetic diversity than freshwater and terrestrial species (Durand and Blanc 1988, Wood 1989, Gray 1997). This tendency has been associated with a stronger gene flow due to the relative absence of barriers to dispersal, and to large effective population sizes in marine environments (Ward et al. 1994). Specifically, biodiversity on coasts has been reported to be particularly high compared to other marine systems, as a result of the great range of environmental fluctuations and niches (Ray 1991).

At a local level, several populations showed no genetic diversity (LP, AC, PA, HA, EC, PV, ES, NA, BR). Null genetic variation might be related to both ecological and anthropogenic factors. Earlier observations indicated that the presence of major and highly polluted river mouths, core industrial and port developments, contaminants (e.g. organic pollutant discharges and heavy metal wastes) and local anthropogenic activities (such as oil industry) negatively alter marine fungal communities in the Gulf of Mexico (Rosales-Hoz et al. 1994, Velez et al. 2013, 2015b, 2016). Genetic diversity in this littoral region is low, perhaps as a result of habitat deterioration and fragmentation, resulting in the reduction of the population size and a consequent increment in variability loss rate through genetic drift (Ellstrand and Elam 1993), in addition to restricted large-scale gene flow resulting from

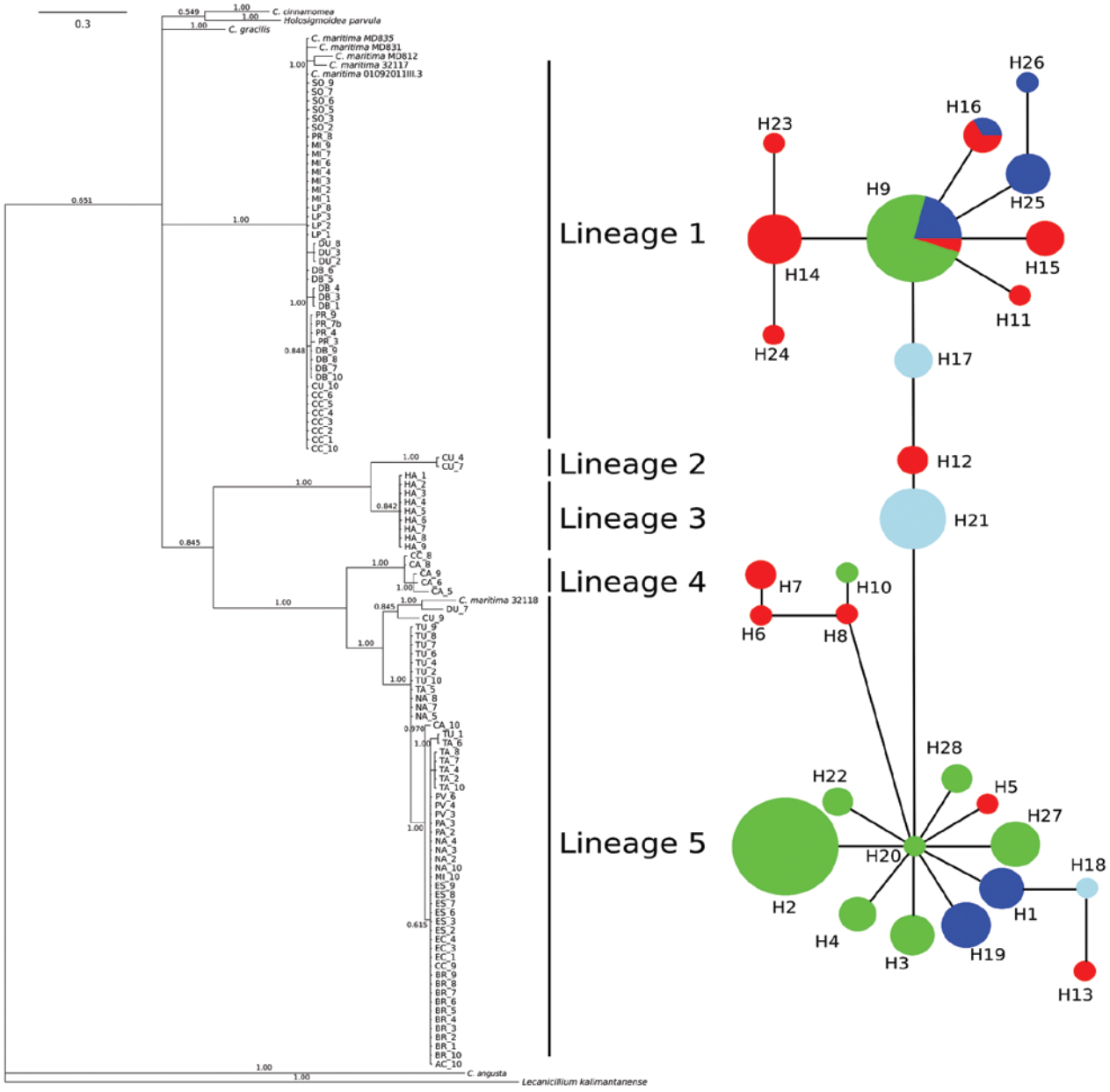

Figure 4: Phylogenetic relationships among the sampled isolates of *Corollospora maritima sensu lato* from locations on several widely separated shores (left), and haplotype network of the five divergent genetic lineages (right). Radius of each circle is proportional to the number of isolates examined, and subgroup of each isolate is indicated by color: green, Gulf of Mexico; dark blue, Eastern Pacific Ocean; light blue, Western Pacific Ocean; red, Caribbean Sea.

sea currents dynamics, such as eddies from the Caribbean Current, which might block the connectivity of the Gulf of Mexico with the Caribbean Sea (Murphy et al. 1999).

On the other hand, the Caribbean population CU displayed the highest genetic diversity. Our findings agree with a previous study on the genetic diversity in some isolates of *C. maritima sensu lato* based on inter-simple sequence repeats (ISSR), which indicated that isolates from the Caribbean Sea harbored high genetic diversity levels (Velez et al. 2016). High levels of genetic variability are considered as a favorable fitness trait for populations, conferring the ability to respond to threats and environmental change (Amos and Harwood 1998). This concurs with previous reports describing the Caribbean Sea as a marine center of speciation and high diversity, as a consequence of intense competition and ecological partitioning, exporting and accumulating genetic lineages generated in peripheral habitats, all leading to biological diversification (Carpenter et al. 2011, Bowen et al. 2013). Additionally, the Bahía de Cochinos on the Cuban coastline represents

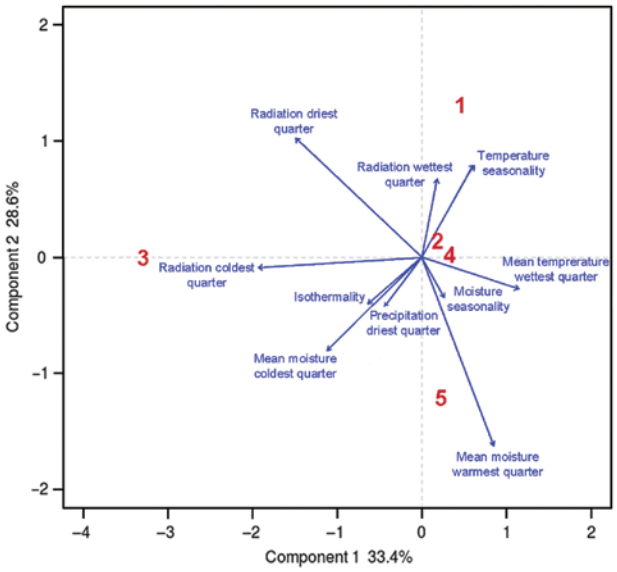

Figure 5: Constrained correspondence analysis ordination biplot showing relationships between environmental factors (blue arrows), and the genetic lineages within *Corollospora maritima sensu lato* (numbers in red). For site nomenclature, refer to Table 1.

a nearly pristine site with well-preserved coastal environments (Caballero et al. 2004), perhaps promoting high diversity levels.

Similar patterns were detected with Tajima's estimate *D*. This estimate provides insights into the evolutionary history of a particular nucleotide sequence, discriminating between neutrally and non-randomly evolving DNA sequences (due to directional selection or balancing selection, demographic expansion or contraction, etc.). The *D* parameter, calculated as the difference between the mean number of pairwise differences and the number of segregating sites, suggests that *C. maritima sensu lato* populations might have suffered either a recent bottleneck, or overdominant selection. This tendency was further observed in the Eastern Pacific Ocean and the Gulf of Mexico regions, which comprise highly polluted and deteriorated sites. However, in the Western Pacific Ocean and the Caribbean Sea regions, the low values of *D* suggest that population sizes are either increasing or are subject to the effect of purifying selection (Hedrick 2011). Therefore, a conservation genetics approach to assess this matter is urgent, especially in strongly impacted beaches where valuable genetic diversity is negatively affected as a result of human-related activities.

Population size is strongly related to genetic diversity (mainly through mutation and gene flow), with smaller populations carrying less variability (lost via genetic drift and selective pressures; Amos and Harwood 1998, Charlesworth and Charlesworth 2010). Similarly, the effective population size (N_e, the size of an ideal population with the same increase in homozygosity and the same random drift in allele frequencies as the actual population considered) plays a key role in genetic diversity modeling (Fisher 1930, Wright 1931, Crow and Kimura 1970, Ewens 1990). In addition, high genetic diversity levels have been also associated with species inhabiting unstable environments on an evolutionary time scale, although these conditions have been proved to reduce genetic diversity on an ecological time scale (Nevo et al. 1984; Alberte et al. 1994). In summary, the contrasting diversity levels in the populations of *C. maritima sensu lato*, are the result of the interaction among several factors such as population size, N_e, wide geographical distribution (distinctive local conditions confer particular selective pressures), and fluctuating environmental conditions (typical of ecotones) and perhaps anthropogenic pressures.

Genetic structure

The cluster analysis based on genetic distances revealed local geographic patterns in some neighboring populations of the Gulf of Mexico and the Eastern Pacific Ocean. Similarly, the consistent clustering of SO and LP resembles the trajectory of superficial sea currents in the Gulf of California (Pantoja et al. 2012). Nevertheless, large-scale geographical patterns were not clearly detected, since sites from widely separated shores grouped together. Moreover, Caribbean populations showed the greatest geographical incongruence. These results agree with ISSR data, where phylogeographic patterns were not clearly detected, perhaps as a result of the geographical co-occurrence of several genetic clusters (Velez et al. 2016).

Consistently, the phylogenetic reconstruction identified five genetic lineages with an overlapping phylogeographic distribution. The first lineage encompassed isolates from the Eastern Pacific Ocean, Gulf of Mexico, Western Pacific Ocean, and Caribbean Sea; the fourth comprised isolates from the Gulf of Mexico, and the Caribbean Sea; and the fifth included isolates from the Gulf of Mexico, the Caribbean Sea, Western Pacific Ocean, and Eastern Pacific Ocean. As these results demonstrate the geographical co-occurrence of several genetic lineages in some sites of the Caribbean Sea, Gulf of Mexico and Western Pacific Ocean, they might explain the lack of genetic structure among populations.

The haplotype network showed that some haplotypes were restricted to occur not only at a lineage level, but also at a littoral level perhaps as a result of local selective

forces, and genetic drift, leading to fixation. For example, the genetic lineages 2 and 3 were represented by the private haplotypes H12 (from the Caribbean Sea) and H21 (from the Western Pacific Ocean), respectively. Haplotype H7 (restricted to the Caribbean Sea) was the most dominant in lineage 4. Finally, the genetic lineage 5 was dominated by H2, which is restricted to the Gulf of Mexico.

We also detected H9 from the genetic lineage 1 as a widely distributed and dominant haplotype in populations from the Gulf of Mexico, Caribbean Sea and Eastern Pacific Ocean, suggesting gene flow among widely separated shores. Previous studies suggest the possible transatlantic dispersal of ascospores in *Corollospora maritima sensu lato*, since they possess specialized morphology (i.e. thin and sheet-like polar and equatorial appendages) which aid attachment to dispersal vectors such as marine animals, driftwood, ships, marine migratory birds, among others (Kohlmeyer 1968, Kohlmeyer and Kohlmeyer 1979, Velez et al. 2016). Furthermore, this phenomenon might be enhanced by storms and hurricanes, which represent natural disruptions often occurring in marine ecosystems (Kohlmeyer and Kohlmeyer 1979, Jones et al. 2006).

Environmental affinities

In the present study we evaluated isolates from temperate and tropical sandy beaches in the biogeographic provinces designated by Bebout et al. (1987). Our CCA results resemble prior observations by these authors on the differential growth of some isolates of *Corollospora maritima sensu lato* in response to temperature, yet temperature does not represent an exclusive variable explaining the geographical distribution of genetic diversity. Through the CCA highlighting correlations between genetic lineages and some environmental variables, we identified the differential influence of mostly temperature, radiation and moisture-related environmental variables on the allocation of most of the genetic lineages within *C. maritima sensu lato*.

Genetic lineage 1 was positively associated with seasonal temperature and solar radiation in the wet quarter. This lineage includes temperate and tropical sites from the Caribbean Sea, Eastern and Western Pacific Ocean, and perhaps is characterized by its broad tolerance for temperature fluctuations. Lineage 3 was linked to the solar radiation in the cold quarter, implying narrower environmental restrictions associated with cooler temperatures, whereas lineage 5 was correlated with the mean moisture during the warm quarter. Lineage 5 comprises sites in the Eastern and Western Pacific Ocean, Gulf of Mexico and Caribbean Sea, suggesting a strong environmental affinity for humidity over temperature. Remarkably, lineages 2 and 4 (comprising unique populations in the Caribbean Sea) did not show a clear preference for any of the tested environmental variables. Possibly, these genetic clusters are linked to variables denoting the typical ecosystem processes in the Caribbean Sea, such as competition and ecological partitioning (Carpenter et al. 2011, Bowen et al. 2013). Therefore, further factors and larger population numbers must be evaluated to fully explain the geographic distribution of these lineages.

Conclusions

The present study has unveiled the remarkably high levels of genetic variation in *Corollospora maritima sensu lato*, although a clear population structure was not detected. Excessively high genetic levels might be associated with the co-occurrence of genetic lineages in the same site, whereas populations with null genetic diversity might be linked to ecological and anthropogenic factors and small population sizes as well as the presence of a sole genetic lineage. Moreover, the co-occurrence of the dominant haplotype H9 in the Gulf of Mexico, Caribbean Sea and Eastern Pacific Ocean suggests gene flow among widely separated shores. Our results indicate the existence of five genetic groups in the analyzed *C. maritima sensu lato* isolates from several sandy beaches in the Pacific Ocean, Caribbean Sea and Gulf of Mexico. Based on the results discussed above, we suggest that the cosmopolitan classification of *C. maritima sensu lato* should be carefully reexamined, especially at the population level, since our results indicate that several genetic lineages co-occur in some locations (Bebout et al. 1987, Amos and Harwood 1998).

Acknowledgments: This research was supported by the Institute of Biology, National Autonomous University of Mexico, and by the Consejo Nacional de Ciencia y Tecnología (CONACyT), project number CB 60502. Patricia Velez also thanks the fellowship provided by the CONACyT. The authors recognize the help provided during the fieldwork in the Mexican states of Tabasco, Campeche and Veracruz by Edmundo Rosique-Gil and Silvia Capello from the División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, as well as Sergio Martínez from the Centro de Biodiversidad y Descubrimiento de Drogas, INDICASAT-AIP, Xavier Alvarez from the Facultad de Ciencias Naturales, Universidad de Guayaquil, staff of the Instituto de Oceanología, Christian Quintero and Jan Hanlin for the assistance in the sample collection of Panama, Ecuador,

Cuba, Sonora State in Mexico, and Hawaii, respectively. We would also like to extend our sincere appreciation to anonymous reviewers for their valuable comments.

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Supplemental Material: The online version of this article (DOI: 10.1515/bot-2016-0058) offers supplementary material, available to authorized users.

Bionotes

Patricia Velez
Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-233, Mexico City 04510, Mexico,
pvelezaguilar@gmail.com.
http://orcid.org/0000-0002-4449-8977

Patricia Velez earned her PhD in Fungal Ecology from the National Autonomous University of Mexico in 2014 for her work on the community structure of marine arenicolous fungi from the Gulf of Mexico and the population genetics of *Corollospora maritima*. She has previously worked on the comparative transcriptomics of this cosmopolitan marine fungus, and in the fungal ecology of an oligotrophic freshwater system. Currently she is a postdoctoral researcher at the Centro de Investigación Científica y Educación Superior de Ensenada (CICESE) and her work focuses on the molecular ecology of marine fungal communities from deep-sea sediments.

Jaime Gasca-Pineda
Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, Mexico City 04510, Mexico

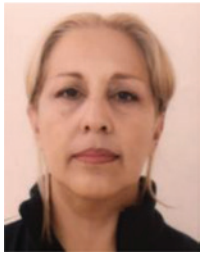
Jaime Gasca-Pineda is currently a postdoctoral researcher at the Centro de Investigación Científica y Educación Superior de Ensenada (CICESE). He received his PhD in Biomedical Sciences from the National Autonomous University of Mexico in 2015 for his work on the population genetics of bighorn sheep and bison from Mexico. He has also conducted research on the molecular ecology of wild maize and microbial communities. More recently, his postdoctoral research has focused on the population genomics of ichthyoplankton from the Gulf of Mexico.

Akira Nakagiri
Division of Genetic Resource Preservation and Evaluation, Fungus/Mushroom Resource and Research Center, Tottori University, Tottori 6808553, Japan

Akira Nakagiri is a Professor at the Faculty of Agriculture, Fungus/Mushroom Resource and Research Center, University of Tottori. In 2014, he received an award from the former Japan Society for Culture Collections (current Japan Society for Microbial Resources and Systematics) for his contributions to fungal taxonomy and to the preservation of fungal cultures. His research areas range from fungal taxonomy and ecology of marine taxa to the preservation of fungal cultures. He is a specialist in marine fungal taxonomy and biology, having described several new taxa including members of the genus *Corollospora*.

Richard T. Hanlin
Mycology Herbarium, Museum of Natural History Annex, University of Georgia, 4435 Atlanta Highway, Athens, GA 30606, USA

Richard T. Hanlin, Professor Emeritus at the University of Georgia, retired in 2001 after 41 years in the Department of Plant Pathology. He continues to serve as a part-time curator of the Miller Mycology Herbarium in the Museum of Natural History. He is a member of the Mycological Society of America, served as vice president and president, and is a recipient of the society's W. H. Weston Award for Teaching Excellence in Mycology and Distinguished Mycologist Award.



María C. González
Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-233, Mexico City 04510, Mexico

María C. González received her DSc from the National Autonomous University of Mexico. She is presently a permanent researcher at the Institute of Biology at the same university, where she teaches mycology in graduate level courses and conducts research on the biology of microscopic fungi from freshwater and marine habitats in Mexico.