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A Morphometric and Geo Morphometric Study Comparing Two Sub Species of *Taphrina Caerulescens* and *Taphrina Deformans*

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Abstract

Taphrina caerulescens has been poorly studied although it was known about since the beginning of the past century. One of the only studies which seek to describe morphometrically differences among the species of Taphrina ``A monograph of the genus Taphrina`` by A.J Mix is out dated and was published in 1949. In the study Mix states that there is little or no differences between Taphrina caerulescens which up until now was not thought to be present in Mexico and which causes blisters exclusively on *Quercus* spp. And Taphrina deformans which causes blisters on leaves and fruit of peaches and cherries and a few other stone fruit producing species. Mix in his monograph also stated that there is a fair amount of morphometrically difference of the asci of *T. caerulescens* depending on which *Quercus* spp that it infects and this difference is due to the different types of sources of carbohydrates that the fungus metabolizes. In this study images of the different asci were mapped into coordinates and a TPS file was created, each image was transformed to 107 landmarks. The TPS files was processed (the images were Procrustes fitted) using the TPS utility program version 1.70, Relative warps version 1.65 and TPSDig2 Version 2.26 software packet by Rohlf 2001. The data generated was further analyzed using the Past 3.14 software. The analysis showed that there was no difference between the two *Taphrina* sub species isolated from *Quercus eduardii* and *Q. potosina*, however they were significantly different to T. deformans.

Keywords: *Taphrinadeformans; Taphrinacaerulescens*, Procrustes, TPS, *AscusQuercus eduardii, Quercus potosina*

Introduction

Figure 1: Leaves of *Q. eduardii* with typical symptoms of leaf blister.

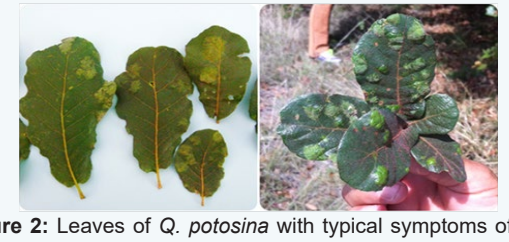

Figure 2: Leaves of *Q. potosina* with typical symptoms of leaf blister.

Figure 3: Leaves and fruit of *P. persica* with typical symptoms of leaf curl.

Oak leaf blister caused by *Taphrina caerulescens* (Figure 1&2) is endemic to North America. In 2015 in a study of the phytosanitary status of the Sierra Fría of Aguascalientes symptoms very similar to those that are caused by *Taphrina* were observed on numerous oak species by Moreno *Taphrina* diseases are best known in Europe and North America. T*aphrina deformansthe* most notorious species of this genus produces the deformation of leaves and fruits and later the defoliation of the

peaches (Figure 3), which results in the production of smaller fruits and fruit fall. When the disease is severe, it can result in a loss of 50% or more of the fruits. The disease can also affect the buds and twigs of plum and peach, resulting in the weakening of these fruit trees. The disease is more severe in the southeastern Gulf of the United States of America Sinclair et al. [1].

T. caerulescens is closely related to *Taphrina deformans*, which causes blisters on leaves and peach fruits, the asca of these two pathogens are indistinguishable, however, *T. deformans* infects species of peach trees whereas *T. caerulescens* only infects the Oaks The most important economic losses, however, are those produced by *Taphrina deformans* in peach, almond, nectarine and sometimes on plum. Many investigators claim that there is no difference between the ascus formed by *T. caerulescens* in oak species and *T. deformans* which affect prune and other stone fruits.

Little work has been done to confirm the similarly of differences that exist within the species and between the different species of *Taphrina*. In this study we propose the following objectives

I. Difference between the asci of *T. caerulescens* and *T. deformans*

II. Difference between the asci of T. caerulescens taken from leaves of *Quercus potosina* and *Quercus eduardii*

III. And finally compare the three groups

Matrials and Methods

Study area and sample collection-

Leaves of infected *Quercus eduardii* and *Quercus potosina* (Figure 1 & Figure 2) were collected in the Sierra Fria of Aguascalientes, Mexico. The Sierra Fria is located to the in the North Western part of

Aguascalientes within the municipalities of San Jose de Gracia, Calvillo, Rincón de Romos, JesúsMaría and Pabellón de Arteaga; it falls between the following coordinates Latitude N: 21° 52' 45'' a 23° 31' 17'' y Longitude W: 102° 22' 44'' a 102° 50' 53'' and covers an area of 112,090 hectares of mountains and Pine Oak and cedar the maximum elevation of the Sierra Fria is 3050 meters. The predominant fauna is whitetail deer, puma, wild boar, pumas, gray fox, royal eagle, peregrine falcon, quail, chameleon and rattlesnakes SEDESO [2] & Sosa Ramirez et al. [3].

Selection of samples

Samples of asci of *Taphrina caerulescens* were isolated from leaves of *Q potosina* and *Q eduardii* and were selected at random from prepared semi-permanent and permanent slides of leaves infected with the disease. The sample photographs of *Taphrina deformans* Asci (Figure 4) were downloaded from trusted websites; the photos downloaded were also downloaded randomly.

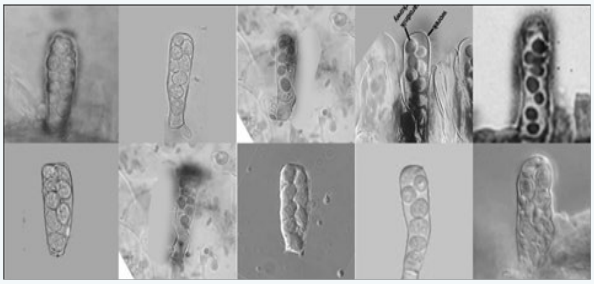

Figure 4: Microscopic images of asci of *T. deformans*.

Asci geometric morphometry

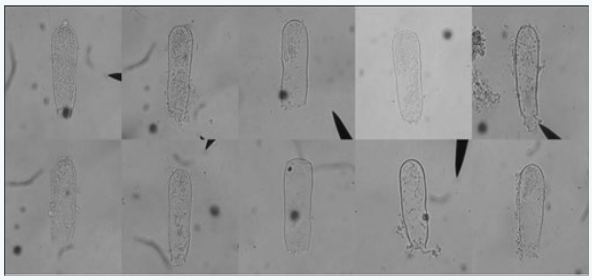

Figure 5: Microscopic (40x) images of asci of *T. caerulescens* isolated from *Q. eduardii*

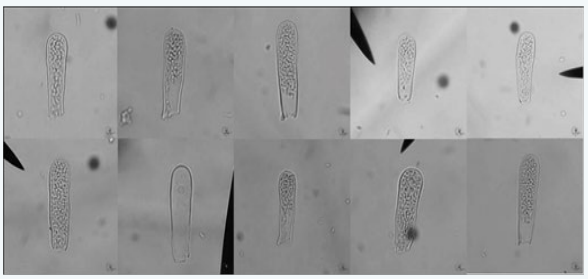

Figure 6: Microscopic images (40x) of asci of *T. caerulescens* isolated from *Q. potosina*.

Photographs of ascus (Figure 5&6) were edited using the Photoshop software package and were photographed using a Nikon D3000 which was mounted on a compound microscope (Leica DMS) at a magnification of 400x. After images were processed the configurations of landmark coordinates were scaled, translated and rotated by a generalized Procrustes analysis (GPA) using MorphoJ, TPSutil, TPS relw and TPS dig programs Klingenberg (4) & Rohlf [5]. A total of 107 landmarks were used per image.

Data analysis and statistic

Multivariate Analysis of Variance (MANOVA) Rohlf [5] was used to explore individual elemental fingerprint differences between the different isolates analyzed, followed by the Paired hoteling test to further test for differences between the isolates collect from the two oak species. Previously, normality and homogeneity of variance were tested (Shapiro-Wilk test, $p > 0.05$ and Shapiro-Wilk test, $p > 0.05$, respectively).

This geometric analysis was performed using 107 landmarks, reconstructed from distance measurements among

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the landmarks. Shape variables generated from the landmark analysis were considered to be invariant regarding mathematical differences in translation, rotation, and scale Márquez et al. [6]. The multivariate regression of shape; size was computed as centroid size (CS), the square root of the sum of squared distances from each landmark to the specimen's centroid Loy et al. [7]. The relative warps (RW) were used to construct a matrix and a PCA was performed (relative warp analysis, RWA) in order to describe major trends in shape variations Márquez et al. [6] & Zelditch et al. [8].

Results

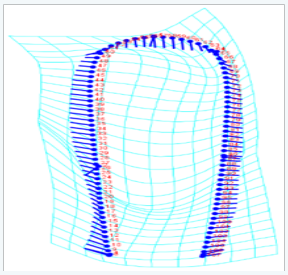

Figure 7: Results of TPS plotting of coordinates of *P. persica* after Procrustes fitting.

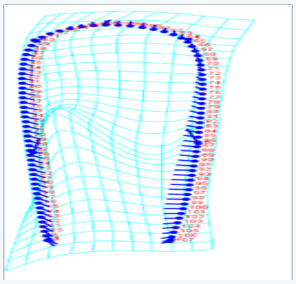

Figure 8: Results of TPS plotting of coordinates of *T. caerulescens* isolated from, *Q. potosina* after Procrustes fitting.

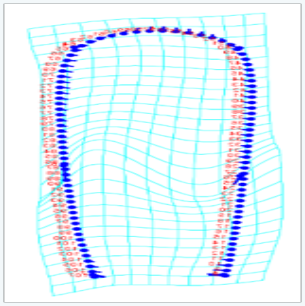

Figure 9: Results of TPS plotting of coordinates of *T. caerulescens* isolated from, *Q. eduardii* after Procrustes fitting.

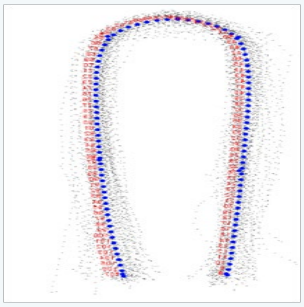

Figure 10: Average of coordinates of the results of TPS plotting of coordinates of all samples after Procrustes fitting.

The results for the images obtained from the TPs subjected to Procrustes fittings (Figure 7-9) shows that although all images follow the same pattern, there is notable more deformation in the *Taphrina deformans* image when compared to the other samples to the average image (Figure 10). This visual analysis is confirmed for after a MANOVA was done for the three samples ($p > 0.05$) *Q eduardii: Q potosina* (0.091958); $p < 0.05$ *Q eduardii*: *P persica* (0.0005858); ($p < 0.05$) 0.0005858 *P. persicae. eduardii,* ($p < 0.05$) 3.75E-05 *P. persicae: Q. potosina*. Results are displayed in Table 1.

Table 1: MANOVA.

	Quercus eduardii	*Quercus potosina*	*Prunus persica*
Quercus eduardii		0.091958	0.0005858
Quercus potosina	0.091958		3.75E-05
Prunus persica	0.0005858	3.75E-05	

Table 2: PcrScores ofimages after Procrustes fitting.

Id	Host Species	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
0	Quercus eduardii	0.019937	0.0005	0.005429	0.012086	0.008733	-0.00428	-0.00041	-0.0023	-0.00357
1	Quercus eduardii	-0.00967	-0.0073	0.005205	0.012142	-0.00015	0.000865	0.003686	0.000357	-0.00044
2	Quercus eduardii	-0.00366	-0.0025	0.011072	-0.00352	0.006067	0.003732	0.006382	0.004821	-0.00325
3	Quercus eduardii	-0.01658	-0.009	-0.0069	0.009946	0.005442	-0.00523	-0.00109	0.00243	-0.00089
4	Quercus eduardii	0.001231	0.0275	0.01146	0.008196	0.003806	0.014115	-0.00046	-9.80E-05	0.004479
5	Quercus eduardii	-0.00557	-0.002	0.012065	0.001217	-0.00285	0.000869	-0.0011	0.00327	-0.00284
6	Quercus eduardii	-0.01095	-0.005	0.005449	0.004369	0.002449	0.001318	0.003738	0.003763	0.003582
7	Quercus eduardii	-0.01889	0.0099	0.032575	0.010417	0.001565	-0.0013	-0.01061	-0.00056	-0.00101
8	Quercus eduardii	-0.02674	0.0078	0.000543	2.12E-05	-0.00959	0.002948	-0.00478	0.003034	-0.00063
9	Quercus eduardii	0.01325	0.0165	0.006261	0.00281	-0.00015	-0.0025	0.001623	-0.002	0.002009

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10	Quercus eduardii	-0.01049	-0.0007	-0.00095	-0.01397	0.00725	0.00097	0.001455	-0.00428	-0.00414
11	Quercus eduardii	-0.01654	-0.0012	0.000572	0.005774	0.005194	0.004336	-0.01221	-0.00306	-0.00185
12	Quercus eduardii	-0.01502	-0.0035	-0.00243	0.000727	-0.00494	-0.00571	0.002111	0.001714	0.001441
13	Quercus eduardii	-0.00769	0.0139	0.004065	0.002328	0.009125	0.005931	0.002407	-0.00149	0.000896
14	Quercus eduardii	-0.00986	0.0241	0.005098	-0.00649	0.005125	-0.003	0.000611	-0.0006	0.001145
15	Quercus eduardii	0.00429	0.0113	-0.01396	-0.00621	-0.00323	0.000582	-0.00326	-0.00339	0.00142
16	Quercus eduardii	-0.0014	-0.0118	-0.0026	-0.0067	-0.00023	0.004185	-0.00849	0.000109	0.001495
17	Quercus eduardii	-0.01607	0.01	0.003813	0.001979	-0.00615	0.003633	-0.00237	-0.0024	-0.00054
18	Quercus eduardii	0.00131	-0.005	-0.00078	0.005709	0.00143	0.001768	-0.00054	0.003625	0.000704
19	Quercus eduardii	0.011479	0.0113	0.006815	-0.01837	0.005382	0.005161	-0.00376	0.000747	-0.00144
20	Quercus eduardii	-0.01179	-0.0162	0.003438	0.016465	-0.00401	-0.00433	0.000653	0.001303	0.004184
21	Quercus eduardii	-0.01148	0.0004	-0.01172	0.013397	0.001885	-0.00966	-0.00024	0.002933	-0.00011
22	Quercus eduardii	-0.0052	-0.0055	0.000877	0.002078	-0.00883	-0.00321	-0.00156	0.000989	0.001638
23	Quercus eduardii	-0.00861	0.0003	-0.00753	0.009393	-0.00346	0.008189	-0.00316	0.004339	0.002386
24	Quercus eduardii	0.002762	0.0153	-0.00439	-0.00071	-0.00046	0.00984	0.002382	-0.00095	0.001025
25	Quercus eduardii	0.005345	0.0087	-0.00021	-0.00322	-0.00865	-0.00781	-0.00192	-0.0031	0.003026
26	Quercus eduardii	0.006231	0.0017	-0.0112	0.002779	0.001434	-0.00153	0.001059	-0.00322	0.000796
27	Quercus eduardii	-0.0017	-0.0039	0.014923	0.007131	-0.00545	-0.00482	0.004094	0.006117	0.00109
28	Quercus eduardii	-0.00941	-0.0037	-0.00267	0.009438	-0.00511	-0.00243	-0.00322	0.001399	0.000296
29	Quercus eduardii	0.001146	-0.0025	-0.03469	0.010877	0.004527	0.008248	0.002189	0.002328	-0.00211
30	Quercus potosina	0.001253	0.0172	-0.00178	-0.01647	0.001966	-0.00311	0.003203	0.001689	0.000255
31	Quercus potosina	-0.00825	-0.0081	-0.00714	0.014242	-0.00517	-0.00435	-0.00266	-0.00603	-0.00164
32	Quercus potosina	0.00833	0.0185	0.003365	-0.00192	-0.00624	-0.00066	-0.00058	-0.00207	0.001605
33	Quercus potosina	0.014472	0.0015	0.001303	0.012461	0.006594	0.000264	-0.00426	-0.00094	-0.00175
34	Quercus potosina	0.006302	0.0066	-0.00936	-0.00808	-0.0029	-0.00592	0.00032	-0.00021	-0.00026
35	Quercus potosina	0.009403	0.0087	0.011666	0.005931	-0.0047	-0.00361	0.003373	-0.00161	0.001919
36	Quercus potosina	0.000782	0.0039	0.006231	0.008298	0.001003	0.006427	0.000508	-0.00597	-0.00011
37	Quercus potosina	0.018147	0.0158	0.001405	-0.01495	0.004981	-0.00744	-2.20E-05	-0.0008	-0.00174
38	Quercus potosina	0.003552	0.0185	-0.02237	-0.0063	-0.00833	-0.01182	0.001537	-0.00225	-0.00251
39	Quercus potosina	0.023557	-0.004	0.003107	0.011475	0.008672	-0.00695	-0.0013	-0.00094	0.001133
40	Quercus potosina	0.009543	-0.0084	-0.00618	0.003036	-0.01121	-0.00705	-0.00412	-0.00406	0.002897
41	Quercus potosina	0.007539	0.0155	0.004706	0.005093	0.009137	0.001423	-0.00418	-0.00064	-0.00371
42	Quercus potosina	0.00162	-0.0185	-0.0175	-0.00686	-0.00283	-0.00348	0.001108	0.003575	-0.0034
43	Quercus potosina	0.02328	0.0095	0.02274	-0.00753	-0.00239	-0.01032	0.006614	0.00372	-0.00066
44	Quercus potosina	0.01857	-0.0066	0.00546	0.001714	0.002987	-0.01166	0.001355	-0.00031	-0.00013
45	Quercus potosina	0.010971	0.0141	0.000956	-0.00342	0.0009	-0.0027	0.00567	-0.0022	-0.0014
46	Quercus potosina	0.003095	0.0224	-0.00414	-0.01227	0.001161	0.00317	-0.00375	0.004443	-0.00043
47	Quercus potosina	0.024539	0.0145	-0.00596	0.002044	0.004843	-0.00682	0.000805	-0.0006	0.000183
48	Quercus potosina	0.009341	0.0197	-0.00221	0.013898	-0.00107	0.005633	-0.00026	0.003965	0.001784
49	Quercus potosina	-0.00207	0.0023	-0.01909	0.004287	-0.00699	0.004326	-0.00418	0.002772	-0.0031
50	Quercus potosina	0.003109	0.0084	0.000274	-0.00474	-0.00205	-0.00324	-0.0036	-3.52E-05	-0.00013
51	Quercus potosina	0.033936	-0.0108	-0.0102	0.00201	-0.00074	0.008785	0.000135	0.006068	-0.0005
52	Quercus potosina	0.015004	-0.0175	0.007143	0.023237	0.014112	-0.00193	0.006169	-0.00091	-0.0009
53	Quercus potosina	-0.02116	-0.0059	0.027932	-0.00974	0.008887	-0.00839	-0.0008	0.005197	0.000249
54	Quercus potosina	-0.00418	0.013	0.005453	0.00067	-0.00173	0.003238	-0.00058	-0.00078	0.003924
55	Quercus potosina	-0.00293	0.0026	0.001094	0.016721	0.004742	0.003991	0.001462	0.003934	-0.00019
56	Quercus potosina	0.00824	0.0023	-0.01066	0.00033	0.000785	0.006817	0.000172	-0.0045	0.002128

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57	Quercus potosina	0.017456	-0.0262	-0.0109	0.007046	-0.00455	-0.00698	-0.00616	0.001565	0.002242
58	Quercus potosina	0.013002	0.0256	-0.01078	-0.00957	0.000517	0.005559	-0.00186	0.000782	0.001476
59	Prunus persica	-0.04049	0.0155	0.026917	-0.00088	-0.00343	-0.00027	0.006176	-0.00105	0.000909
60	Prunus persica	-0.02302	0.0043	-0.00692	-0.02092	0.005127	0.002466	-0.00377	0.000874	0.000163
61	Prunus persica	-0.0054	-0.003	0.015607	-0.01516	0.003029	0.000999	0.004326	-0.0015	-0.00151
62	Prunus persica	0.008282	0.0139	-0.00945	-0.00359	-0.00976	0.013292	0.00417	0.001052	0.000928
63	Prunus persica	-0.02127	-0.0221	-0.01209	-0.00682	-0.00339	-0.0053	-0.0032	0.000105	-0.00208
64	Prunus persica	0.023745	-0.0115	-0.00116	0.002848	0.00202	0.010953	0.004774	0.003886	-0.00192
65	Prunus persica	0.037302	-0.0429	0.006221	0.005498	-0.00291	0.003434	0.00118	-0.00294	0.001083
66	Prunus persica	-0.00352	-0.048	0.007952	-0.02843	-0.00677	0.001298	0.000872	0.002356	0.001031
67	Prunus persica	-0.00196	-0.0208	0.02104	-0.00348	-0.00156	0.007518	-0.00759	-0.00654	0.0001
68	Prunus persica	-0.02337	-0.0362	-0.02651	-0.01652	0.025706	0.004448	0.000709	-0.00156	0.00744
69	Prunus persica	0.003991	0.0002	-0.00347	-0.00467	-0.01192	0.000652	0.004254	-7.44E-05	0.000887
70	Prunus persica	8.74E-05	-0.0096	-0.01335	0.00169	-0.00938	0.007926	0.009663	-0.00547	-0.00488
71	Prunus persica	0.022581	-0.0028	-0.00016	-0.0174	-0.00459	-0.0065	-0.00277	0.003	0.00264
72	Prunus persica	-0.00563	-0.0011	-0.00869	-0.00933	-0.00039	0.008292	-0.00212	0.000115	-0.00471
73	Prunus persica	0.01323	-0.0162	0.013792	-0.00847	-0.00043	0.002692	0.00728	-0.00588	0.002853
74	Prunus persica	-0.02912	-0.0248	0.018439	0.005043	-0.01232	0.01032	0.005353	-0.00084	-0.00257
75	Prunus persica	-0.01433	0.0059	-0.02583	-0.00156	0.00284	-0.00835	0.003139	-0.00029	8.36E-05
76	Prunus persica	0.025307	-0.0169	0.023604	-0.01024	0.004255	-0.00039	-0.00596	-0.0005	-0.00375
77	Prunus persica	-0.0198	-0.0019	-0.00854	0.009633	0.001194	-0.00971	-0.00388	9.30E-06	-0.00212
78	Prunus persica	-0.04272	0.0041	-0.01161	0.002017	0.006109	-0.00785	0.010062	-0.00346	-0.00058

The difference between the samples is also seen when the main principal components were plotted on a graph (Table 2) The Paired Hoteling however showed a slight difference between the samples taken from the two oak species (T^2:35.786); (F:2.4283); (p :0.046105) see results in Table 3.

Table 3: Paired hoteling test between the isolates of *Quercus potosina* and *Quercus eduardii*.

T^2	35.786
F	2.4283
df1	10
df2	19
p	0.046105

Discussion

Results the geometric morphometric indices showed high separation between the ascus of *T. deformans* and *T. caerulescens*. This is very visible and very obvious in the graph where the main principal components are plotted. However, the results also show some degree of separation between the two isolates of *T caerulescens*, these results are in keeping with the already published works of other investigators principally Mix in hoes monograph of the Genera *Taphrina*. It is however important to determine the analytical tools to be used in these types of experiment.

when the PCR scores were analyzed using MANOVA it only showed that the *T caerulescens* isolates differed significantly when compared to *T deformans*, this test however did not show differences between the *T. caerulescens* isolates, however on comparing the *T. caerulescens* isolates with the Paired Hoteling (p :0.046105) it did show some level of separation.

This means that it would be possible perform monitoring studies without the need to apply the multiple techniques to show separation, to show differences among isolates of the same species the paired Hoteling method can be used alone. And to identify differences among various species a MANOPVA analysis of the PCR scores can be successfully used. This can help reduce the duplication of work, effort and possibly the cost in some cases; this is in keeping with observations made by Aviglianoa et al. [9].

In conclusion, this study is the first part in refuting other studies which claim that there are no differences between the ascus of *T. deformans* and *T. caerulescens* it also partially supports the claims that although there is some lever of separation, among the isolates of *T. caerulescens* they are still relatively similar in terms of their shapes. More study is recommended however to compare more isolates of *Taphrina caerulescens* on other host oak species of the Sierra Fria, Aguascalientes [10].

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