Genetic diversity of native *Pinus sylvestris* L. of Gerês accessed by SSR markers (MICROSAT – PSYLV)

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Introduction – 
*P. sylvestris* distribution

This distribution map showing the natural distribution area of *Pinus sylvestris* in Europe was compiled by members of the EUFORGEN network.

Citation: Distribution map of Scots pine (*Pinus sylvestris*). EUFORGEN 2008. www.euforgen.org.

Post published online on September 2004 - Updated on 24 July 2005
Introduction –

*P. sylvestris* in Portugal

- Data suggests the presence of *Pinus* sp., including *P. sylvestris*, thought the center and north of Portugal during the Last Pleistocene and Holocene.

- Most of the *P. sylvestris* populations existent in Portugal resulted from afforestations performed in the beginning of the XX century.

- *P. sylvestris* populations present at Bidúiça (Bid.) and Ribeira das Negras (R.N.) - Serra do Gerês, NW Portugal - were firstly described at 1913 as autochthonous populations.

- Bid. and R.N. are located at small areas, at the bottom of valleys and near watercourses.
Objectives:

- Characterize the genetic structure and diversity of Biduiça and Ribeira das Negras populations with SSR markers

- Support or disavow the hypothesis of the existence of native *P. sylvestris* populations in Portugal
Sample Collection

- Biduíça – 30 ind. (needle)
- Ribeira das Negras – 20 ind. (needle)
- Puebla de Lillo – 10 ind. (needle)
- Montes Universales – 10 ind. (needle)
- Germany – 10 ind. (needle)
- Áustria – 16 ind. (seed)
Methodology

Genomic DNA Extraction
-> PCR & Capillary Electrophoresis (cpSSR and nSSR)
-> Results
-> Alelle Binning (Allelograme)

<table>
<thead>
<tr>
<th>DNA</th>
<th>Primer</th>
<th>Repeat</th>
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<tbody>
<tr>
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<td>Pt26081</td>
<td>(T)_{14}</td>
</tr>
<tr>
<td></td>
<td>Pt30204</td>
<td>(A)<em>{12}(G)</em>{10}</td>
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<tr>
<td></td>
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<td>(T)_{14}</td>
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<td>(T)_{16}</td>
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<td>Pt51569</td>
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<td>(AT)<em>{5}(GT)</em>{19}</td>
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<td></td>
<td>SPAC11.8</td>
<td>(TG)_{16}</td>
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<td>SPAC12.5</td>
<td>(GT)<em>{20}(GA)</em>{10}</td>
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<td></td>
<td>SPAG7.14</td>
<td>(TG)<em>{17}(AG)</em>{21}</td>
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### Chloroplast Microsatellites

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<tr>
<th>Populations</th>
<th>N</th>
<th>A</th>
<th>P</th>
<th>Nh</th>
<th>Rh</th>
<th>Hg</th>
<th>D^2_{sh}</th>
<th>Hs</th>
<th>Dst</th>
<th>Ht</th>
<th>F_{stc}</th>
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</thead>
<tbody>
<tr>
<td>Biduíça</td>
<td>30</td>
<td>14</td>
<td>11</td>
<td>5.422</td>
<td>5.274</td>
<td>0.844</td>
<td>3.921</td>
<td>0.255</td>
<td>0.038</td>
<td>0.293</td>
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<tr>
<td>Ribeira das Negras</td>
<td>20</td>
<td>5</td>
<td>4</td>
<td>2.740</td>
<td>2.983</td>
<td>0.668</td>
<td>6.412</td>
<td>0.132</td>
<td>0.041</td>
<td>0.173</td>
<td>0.235</td>
</tr>
<tr>
<td>Puebla de Lillo</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>8.333</td>
<td>8.000</td>
<td>0.978</td>
<td>3.809</td>
<td>0.092</td>
<td>0.014</td>
<td>0.106</td>
<td>0.132</td>
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<td>Montes Universales</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>10.000</td>
<td>9.000</td>
<td>1.000</td>
<td>4.427</td>
<td>0.094</td>
<td>0.013</td>
<td>0.107</td>
<td>0.124</td>
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<tr>
<td>Germany</td>
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<td>10</td>
<td>8</td>
<td>10.000</td>
<td>9.000</td>
<td>1.000</td>
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<td>0.094</td>
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<td>0.107</td>
<td>0.123</td>
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<tr>
<td>Austria</td>
<td>16</td>
<td>15</td>
<td>12</td>
<td>14.222</td>
<td>8.625</td>
<td>0.992</td>
<td>4.118</td>
<td>0.155</td>
<td>0.018</td>
<td>0.173</td>
<td>0.104</td>
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### Nuclear Microsatellites

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<th>Populations</th>
<th>N</th>
<th>N_{an}</th>
<th>N_{en}</th>
<th>I_{n}</th>
<th>F_{n}</th>
<th>H_{gn}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biduíça</td>
<td>30</td>
<td>10.500 ±1.258</td>
<td>5.161 ±0.503</td>
<td>1.861 ±0.051</td>
<td>0.219 ±0.122</td>
<td>0.815 ±0.742</td>
</tr>
<tr>
<td>Ribeira das Negras</td>
<td>20</td>
<td>7.250 ±0.750</td>
<td>4.046 ±0.666</td>
<td>1.573 ±0.161</td>
<td>0.328 ±0.155</td>
<td>0.742 ±0.440</td>
</tr>
<tr>
<td>Puebla de Lillo</td>
<td>10</td>
<td>10.250 ±0.250</td>
<td>7.903 ±0.722</td>
<td>2.181 ±0.058</td>
<td>0.341 ±0.091</td>
<td>0.916 ±0.540</td>
</tr>
<tr>
<td>Montes Universales</td>
<td>10</td>
<td>9.500 ±0.957</td>
<td>6.492 ±1.225</td>
<td>2.000 ±0.166</td>
<td>0.236 ±0.032</td>
<td>0.861 ±0.512</td>
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<tr>
<td>Alemanha</td>
<td>10</td>
<td>8.250 ±1.109</td>
<td>5.892 ±1.074</td>
<td>1.892 ±0.156</td>
<td>0.294 ±0.049</td>
<td>0.857 ±0.510</td>
</tr>
<tr>
<td>Austria</td>
<td>16</td>
<td>12.250 ±1.974</td>
<td>8.301 ±2.125</td>
<td>2.197 ±0.232</td>
<td>0.371 ±0.082</td>
<td>0.878 ±0.511</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>16,000 ±1.523</td>
<td>9.667 ±0.541</td>
<td>6.299 ±0.527</td>
<td>1.951 ±0.070</td>
<td>0.298 ±0.037</td>
<td>0.845 ±0.543</td>
</tr>
</tbody>
</table>
Results

PCA (cpSSR)

A)

PCA (nSSR)

B)
• Bid. and R.N. populations were differentiated from the remaining populations and between themselves

• STRUCTURE was unable to differentiate the foreign populations
Conclusions:

• Biduiça (Bid.) and Ribeira das Negras (R.N.) are genetically different from the remaining European populations

• SSR results support the hypothesis of the existence of native *P. sylvestris* populations in Portugal

• The genetic differences between Bid. and R.N. may be due geographical obstacles and/or gene flow
Publications

**Article paper:**

**Oral Communications:**
Pavia, Ivo; Mengl, Michael; Gaspar, Maria J; Carvalho, Ana; Slunsky, Renate; Jahn, Daniela; Lima-Brito, José; Heinze, Berthold. Evidence of the existence of native Pinus sylvestris L. populations in Serra do Gerês by nuclear and chloroplast microsatellite markers, XXXVIII Jornadas Portuguesas de Genética, Porto, 2013

Pavia, Ivo; Mengl, Michael; Gaspar, Maria J; Xavier, Maria; Slunsky, Renate; Jahn, Daniela; Lima-Brito, José; Heinze, Berthold. Chloroplast microsatellite markers to infer about the probable existence of native Pinus sylvestris population in Portugal", V Jornadas Nacionais de Genética e Biotecnologia, Vila Real, 2013

**Poster Communications:**
Pavia, Ivo; Mengl, Michael; Gaspar, Maria J; Carvalho, Ana; Lima-Brito, José; Heinze, Berthold. Combining Chloroplast and Nuclear Microsatellite Markers to assess two probable native P. sylvestris populations in Portugal, 7TH EPSO CONFERENCE: PLANTS FOR A GREENING ECONOMY, Porto Heli, 2013

**MSc. Dissertation:**
Pavia, Ivo. Molecular and cytogenetic characterization of Pinus sylvestris L. populations. MSc Dissertation in Technological and Comparative Molecular Genetics, University of Trás-os-Montes e Alto Douro, 2013
Recent research:

Molecular extrapolation of the provenances of the actual Portuguese Scots pine populations based on the ITS PCR-RFLP technique

Principal Coordinates Analysis (PCoA)

Coord. 1

Coord. 2

Sweden

Trás-os-Montes
Pedra Bela
Peneda
Serra da Estrela
Vinhais

Ribeira das Negras

Biduissa

Montes

Universales

Pedra Bela

Peneda

Serra da Estrela

Vinhais

Universales

Puebla de Lillo

Coord. 1

Coord. 2

Pavia, I.; Carvalho, A.; Sampaio, A.; Oliveira, C.; Fernandes, C.; Coutinho, J.; Gaspar, M.; Lima-Brito, J. Portuguese Scots pine populations - molecular extrapolation of their origins based on ITS PCR-RFLP markers. Submitted to iForest in May 20th, 2014 (in Revision)
Recent research:
(preliminary results)

Inferences regarding recent genetic history of two Portuguese native *Pinus sylvestris* L. populations: molecular analysis with SCoTs and ISSRs.
Recent research:

Cytogenetic instabilities in metaphase chromosomes of *Pinus sylvestris* L. detected in a potential native Portuguese population

Thank you for your attention