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Combining niche modelling, land use-change, and genetic information to assess the conservation status of *Pouteria splendens* populations in Central Chile

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Abstract

Background: *Pouteria splendens* (lúcumo chileno) is an endemic shrub to the coastal areas of Central Chile classified as Endangered and Rare by the Chilean threatened species list, but as Lower Risk (LR) by IUCN. Based in historical records some authors have hypothesized that *P. splendens* originally formed a large metapopulation, but due to habitat loss and fragmentation these populations have been reduced to two main areas separated by 100 km, neither of both currently protected by the Chilean system of protected areas. Knowledge about this species is scarce and no studies have provided evidence to support the large metapopulation hypothesis. This gap of knowledge limits our availability to gauge the real urgency to conserve remaining *P. splendens* populations, which can generate tragic consequences in light of the increasing land-use change and climatic change that are facing these populations. In this study we combined niche modelling, land-use information, future climatic scenarios, and conservation genetics techniques, to test the hypothesis of a potential original large metapopulation, evaluate the role of land-use change in population decline, assess the threats this species may face in the future, and combine the generated information to re-assess its conservation status using the IUCN criteria.

Results: Our results show that locations with *P. splendens* are fewer than described in the literature. Results from the niche modelling and genetic analyses support the hypothesis of an originally large metapopulation that was recently reduced and fragmented by anthropogenic land-use change. Future climate change could increase the range of suitable habitats for *P. splendens* towards inland areas; however the high level of fragmentation of these new areas is expected to preclude colonization processes.

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Conclusions: Based on our results we recommend urgent actions towards the conservation of this species, including (1) re-evaluating its current IUCN conservation status and reclassifying it as Endangered (EN), and (2) take immediate actions to develop strategies that effectively protect the remaining populations.

Keyword: Habitat loss, Habitat fragmentation, MaxEnt, Genetic diversity, ISSR, Biodiversity conservation, IUCN red list.

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Background

Pouteria splendens (A.DC.) Kuntze (lúcumo chileno, palo colorado) is an endemic shrub of the coastal areas of central Chile, and is the only representative of the Sapotaceae family in Chile (Muñoz & Serra 2006). *P. splendens* is an evergreen shrub, usually no taller than 2 meters, and with an intricate branching physiognomy. However, if conditions are favorable, individuals can develop longer trunks and reach more than 5 meters in height (Hechenleitner et al. 2005).

Historic records for *P. splendens* indicate a distribution that ranged from the coastal areas near the village of Huentelauquén (31°36'24''S; 71°32'10''O) in the north, to the beach resort of Algarrobo (33°22'50''S; 71°40'57''O) to the south, extending for near 200 km along the coastal range of central Chile. However, the current distribution is reported to be restricted to just two main populations isolated from each other by approximately 100 km (Hechenleitner et al. 2005). Among these, the northern area has the larger population, covering an important proportion of the coastal terrace between north of Pichidangui (32°08'19''S; 71°31'46''O) and south of Los Molles (32°14'15''S; 71°30'52''O). The southern area is represented by a few isolated groups of individuals located on coastal cliffs and ravines between the coastal villages of Laguna Verde (33°06"05'S; 71°40"00'O) and Quintay (33°11"38'S; 71°41"56'O).

Current knowledge about *P. splendens* populations is extremely scarce, and until now much of the literature is based on historical presence records and anecdotal reports. For example,

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77 although available literature suggests that current populations are the remnants of a larger
78 metapopulation covering the entire range of the historic distribution (Hechenleitner et al.
79 2005; Muñoz & Serra 2006), at present there is no empirical evidence, besides the historical
80 presence records, that supports this hypothesis. The lack of information regarding *P.*
81 *splendens* is also illustrated by the inconsistencies among authors or institutions in relation
82 to its conservation status. Whereas Squeo et al. (2001) and Hechenleitner et al. (2005)
83 classified the species as critically endangered, the Chilean species inventory system has
84 listed it as endangered (Ministerio de Medio Ambiente de Chile 2014), and the
85 International Union for Conservation of Nature (IUCN) as lower risk/near threatened
86 (International Union for Conservation of Nature 2014).
87
88 Among the main probable factors responsible for the reduction of *P. splendens*
89 geographical range are the loss and fragmentation of original habitat due to the
90 development of coastal resorts, towns and cities that now cover most of the original range
91 of its distribution (Hechenleitner et al. 2005). Alongside habitat loss, the local extinction of
92 large animals capable to disperse *P. splendens* seeds may have reduced the probability of
93 these seeds to find new suitable sites for germination (Henríquez et al. 2012; Sotes et al.
94 2013). Additionally, the increasing frequency of droughts experienced by these areas due to
95 climate change (Quintana 2012; Schulz et al. 2012) may have reduced *P. splendens*
96 germination rates (Hechenleitner et al. 2005; Sotes et al. 2013) and increased its
97 vulnerability to local extinctions due to an increment in fire regimes (Montenegro et al.
98 2004). Climatic conditions are expected to keep changing in all the range of *P. splendens*
99 distribution (CONAMA 2006; Nuñez et al. 2009), which may generate additional burdens
100 for the preservation of remaining populations. Moreover, genetic diversity could decrease

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in fragmented populations as a consequence of genetic drift, inbreeding, bottlenecks, and founder effects, which together with demographic and environmental fluctuations may determine the persistence of populations in the long run (Lammi et al. 1999).

Even though remaining populations are increasingly threatened by human actions, at present none of the *P. splendens* populations have been covered by the Chilean system of national parks and protected areas (SNASPE), nor have they been included by any kind of public or private effective protection or conservation strategies. This lack of action has occurred even though the northern range of *P. splendens* distribution was officially recognized as an urgent site for conservation one decade ago (CONAMA-PNUD 2005).

The few studies available for *P. splendens* have been focused in understanding the species autoecology (i.e., Sotes et al. 2006; Nuñez et al. 2009; Henríquez et al. 2012; Sotes et al. 2013), and until now there have been no studies at larger scales that may provide answers to the original metapopulation hypothesis. This lack of information is not only limiting our understanding about potential causes of the current fragmented distribution of *P. splendens* populations, but also our ability to gauge the urgency to develop conservation efforts.

The combination of spatially explicit ecological tools, such as species niche modelling (e.g., Phillips et al. 2006; Phillips & Dudík 2008; Mateo et al. 2011) and conservation genetics techniques (e.g., Frankham 2005; Freeland 2005) may provide critical information regarding species original distribution. Furthermore, the increasing availability of spatial land-use databases can offer key insights into the main past causes of population degradation, whereas the development of future climate models may provide valuable

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125 information regarding population potential future trends. Combining and evaluating this
126 information should be taken as major task prior to developing conservation strategies for
127 threatened species (Opdam & Wascher 2004).
128
129 In order to reduce the knowledge gap regarding *P. splendens* past and current populations,
130 and to provide insightful knowledge that can be used for developing planning efforts
131 towards its conservation, in this study we had five main objectives: (1) estimate the
132 potential original distribution range of *P. splendens* by using a niche modelling approach,
133 (2) evaluate potential trends of *P. splendens* populations under a probable future climate
134 change scenario, (3) evaluate the role of land-use change in current and future species
135 distribution, (4) assess intra and inter population genetic diversity in order to evaluate the
136 hypothesis of an original large continuous population, and (5) assess its current IUCN
137 conservation status using this generated data.
138
139 **Methodology**
140
141 *Study Area*
142
143 The study area covered the coastal range of North Central Chile (Fig. 1), which is the
144 historical reported distribution range of *P. splendens* (Hechenleitner et al. 2005; Sotes et al.
145 2013). This area can be broadly characterized as having a Mediterranean climate, with
146 colder temperatures and rainy periods during winter months, and warmer and drier
147 conditions during summer period (Luebert & Pliscoff 2006). Annual average precipitation
148 within this area ranges from ~180 mm to ~400 mm in an increasing gradient north-to-south,

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149 but monthly distribution of precipitation follow the same pattern in the whole range, with
150 July being the wettest month and February the driest. Temperatures are relatively similar
151 for the entire study area, with a mean annual temperature of ~13°C, where July is the
152 coldest month (~10.5°C) and January the warmest (~17°C) (Dirección Meteorológica de
153 Chile 2001). Vegetation within this area is characterized by a predominance of shrub
154 species that are directly influenced by oceanic conditions, with only occasional presence of
155 forest patches, mainly in creeks and valleys facing southern slopes (Gajardo 1994).
156
157 *P. splendens population surveys*
158
159 A total of 22 presence data points of *P. splendens* were used in our study. From these, 21
160 points were collected from herbarium records of the Chilean National Herbarium (SGO)
161 and from the University of Concepción Herbarium (CONC). Records were georeferenced
162 and visited on several field trips during the spring of 2010. We also visited other potential
163 sites having the species, basing our search on satellite imaging interpretation and personal
164 communications. A 22nd presence point was registered during the field trip, representing a
165 small population not previously recorded (see supplementary data). In all the sites we found
166 established individuals we did a rough estimation of abundance by a visual prospection in a
167 ~30 minutes walking distance.
168
169 *P. splendens potential habitat modelling*
170

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171 To model the potential habitat distribution of *P. splendens* we used the principle of
172 maximum entropy, which is part of the software MaxEnt (Phillips et al. 2006). MaxEnt
173 estimates the probability of occurrence of the species of interest using presence only data
174 and a group of environmental variables. We decided to use MaxEnt because it has been
175 proven to be one of the best performing modelling methods available (Elith et al. 2006), it
176 is reliable even with a small number of samples (Pearson et al. 2007), and is freely
177 available. All the habitat distribution models were performed using MaxEnt version 3.3.3k
178 (http://www.cs.princeton.edu/~schapire/maxent).
179
180 Environmental variables for modelling were gathered from the WorldClim database. This
181 database consists of a set of 19 global climatic layers generated by the interpolation of
182 global climatic data such as average and seasonal temperature and precipitation (Hijmans et
183 al. 2005). We used the climatic data corresponding to the period from 1950 to 2000, with a
184 resolution of approximately 1 Km^2 (30 arc-sec).
185
186 Considering the level of spatial resolution of the environment data layers, and to avoid
187 pseudo replication, we checked that our 22 *P. splendens* presence records were at least 1
188 km^2 apart. Additionally, to reduce the potential of model over-parameterization (Merow et
189 al. 2013), we performed (Pearson) correlation analysis of the 19 bioclimatic variables,
190 evaluating all pairs of variables with levels of correlation higher than 0.8 ($r > 0.8$), and
191 keeping only those variables that were biologically significant or easier to interpret. The
192 correlation analyses were performed using the software ENMTools version 1.4.4 (Warren
193 et al. 2010, Warren & Seifert 2011). After the correlation analysis only 8 out the 19
194 bioclimatic variables were used for distribution modelling (Table 1).

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We did not use the default configuration provided by MaxEnt for small samples (i.e., auto features) as recent studies have shown that this configuration would not be the most appropriate in some cases (e.g., Merow et al. 2013; Syfert et al. 2013), especially when dealing with a small number of samples (< 20 - 25) (Shcheglovitova & Anderson 2013), as it was in our case. Therefore, to determine the optimal parameters to configure MaxEnt we compared different models with a combination of the "feature class" and "regularization multiplier" parameters recommended when dealing with small number of samples (Shcheglovitova & Anderson 2013). MaxEnt provides different types of restrictions ("feature class") in the modelling stage such as lineal (L), quadratic (Q), product (P), threshold (T) and hinge (H) (Phillips et al. 2006). We used the following combinations of these features: L, H, LQ, LQH. The regularization multiplier values used were based on Warren and Seifert (2011): 1, 2, 5, 10, 15 y 20. All the models were compared using the corrected Akaike information criterion (AICc) (Warren & Seifert 2011), using for this process the software ENMTOOLS version 1.4.4 (Warren et al. 2010). The results suggested that the best combination of parameters were LQ with a regularization multiplier of 1 (see supplementary data).

Once the optimal parameters were determined, we analyzed the performance of the model of choice using the method proposed by Pearson et al. (2007) for models with small a number of samples. The method consists on a jack-knife procedure that removes one presence point from the original dataset (n=22) to subsequently run the model with the remainder (n – 1, 21 points) presence points. This process is repeated with all the available presence data points, generating 22 different models. Later, the model performance was

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219 analyzed using a prediction success rate and a *p* value using the software "pValuecompute
220 v.1.0" provided by Pearson et al. (2007).
221
222 We produced binary maps using two different thresholds to define the suitable vs. non-
223 suitable habitat. We used the "maximum sensitivity plus specificity logistic (MSS)" and the
224 "10 percentile training presence logistic (10PL)" thresholds (values of 0.203 and 0.298
225 respectively). We used these parameters as they are among the two most commonly used
226 thresholds for creating binary suitability maps for species distribution with MaxEnt (e.g.,
227 Liu et al. 2005; Pearson et al. 2007; Contreras-Medina et al. 2010; Vale et al. 2014).
228
229 *P. splendens potential habitat modelling under climate change*
230
231 To predict potential changes in the habitat distribution of *P. splendens* under a hypothetical
232 climate change scenario, we re-projected the model generated in the previous
233 methodological section using a new environmental layer from the period (2041-2060). We
234 used the climatic projections from the General Circulation Model (GMC) from the United
235 Kingdom Meteorological Office Hadley Centre known as HadGEM2-ES (Jones et al.
236 2011). The new climatic layers were downscaled and calibrated (bias corrected) using
237 WorldClim 1.4 as baseline "current" climate (Hijmans et al. 2005). We used an optimistic
238 approach by using the RCP 2.6 scenario of representative concentrations pathways (RCP)
239 of greenhouse gases (Moss et al. 2010). The RCP 2.6 scenario assumes that the global
240 greenhouse emissions will have their maximum concentration between the years (2010 -
241 2020), declining substantially after this period. We used the same bioclimatic variables
242 utilized to build the habitat distribution model under the "current" climatic conditions.

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243 Binary maps for projected future potential distribution were generated using the same
244 thresholds and approaches used to build the current conditions maps.
245
246 *Land Use-Change mapping*
247
248 To estimate the historical land-use change that has occurred within our study area we used
249 the Chilean Forest Inventory (freely available at http://sit.conaf.cl), which is the most
250 comprehensive and recently updated geodatabase of land-use types of Chile (CONAF
251 2011). From this vector-based geodatabase we identified and grouped all the land-use
252 categories that were explicitly indicating a replacement of original natural habitat, including
253 urban areas, industrial areas, mining areas, agriculture lands, and silvicultural lands. All
254 these polygons were grouped under a single category (i.e., land-change) and rasterized in a
255 100 m pixel size grid to create a land-change layer compatible with the Maxent lattice
256 output. This generated layer was then subtracted from the niche modelling layers to map
257 and calculate the extension of habitat loss. Because we did not have an accurate way to
258 estimate the future trends in land-use change, we decide to use a heavily conservative
259 approach to map future potential distribution under land-use change by assuming that
260 current distribution and extension of anthropogenic land-use will remain constant.
261
262 *Genetic diversity of current populations*
263
264 We used Inter Simple Sequence Repeat (ISSR, Gupta et al. 1994; Zietkiewicz et al.1994) to
265 evaluate the level of inter and intra genetic diversity of current *P. splendens* populations.
266 We collected samples of young leaves from a total of 121 individuals from three different

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areas (Table 2). These areas represent the northern, southern and largest populations for which we found individuals, and were analyzed as three different populations for the genetic analysis. All sampled individuals were georeferenced and their leaves frozen in liquid nitrogen and transported to perform DNA extraction and amplification (through PCR) according to Carrasco et al. (2007).

We tested a set of 16 primers (set ISSR 100/8, Biotechnology Laboratory from University of British Columbia, Vancouver), from which five primers were selected to perform the PCR. The basic characteristics of the primers that gave positive results are shown in supplementary material. Amplified genetic material was run by electrophoresis (2% agarose gel) and patterns were visualized and manually recorded (i.e., band presence or absence) for each individual.

We calculated the percentage of polymorphic ISSR loci (P%) and expected heterozygosity (He) (Nei 1973) by using GenAlex 6.4 computer software (http://biology-assets.anu.edu.au/GenAlEx/Welcome.html; Peakall & Smouse 2012). The relative degree of phenotypic diversity was measured using the Shannon's index.

We estimated the genetic differentiation between populations by using AMOVA Φst (Excoffier et al 1992). We also performed a Principal Coordinate Analysis (PCoA) to evaluate the patterns of clustering based on the binary genetic distance displayed by the GenAlex Software (Peakall & Smouse 2012). In order to analyze the genetic relationship among sampled individuals, we used the Structure Software Version 2.1 (http://pritchardlab.stanford.edu/structure.html), which is a method that assigns individuals

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291 from the entire sample to *k* clusters in a way that the Hardy Weinberg equilibrium are
292 maximally explained (Pritchard et al. 2000). We used Evanno et al. (2005) methodology to
293 estimate the *k* number of clusters by the Structure Software, using a threshold of 0.8 as the
294 membership probability value (*Q*) to assign individuals to a specific cluster.
295
296 *Assessment of the conservation status of P. splendens according to IUCN criteria*
297
298 We used the IUCN Red List Categories and Criteria version 3.1 that includes five criteria to
299 assess the species level of threat (International Union for Conservation of Nature 2001).
300 The criteria are: (a) reduction in population size; (b) small geographic range; (c) small
301 population size and decline; (d) very small or restricted population; and, (e) quantitative
302 analysis of extinction risk. We combined existing information with the data generated in
303 our study to re-evaluate the conservation status of *P. splendens*.
304
305 **Results**
306
307 *P. splendens population surveys*
308
309 After the field campaigns we were able to confirm the current presence of *P. splendens* in
310 only nine sites, which were aligned to the species distribution range reported in the
311 literature. From these, eight corresponded to sites previously reported to contain the
312 species, and one to a site not recorded before. The northern and southern sites corresponded
313 to relatively small and isolated populations (<100 individuals), whereas the sites reported
314 between Pichidangui and Los Molles corresponded to an apparently relatively large

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(thousands of individuals) and continuous population (see supplementary material for sites information).

P. splendens potential habitat modelling

The most relevant bioclimatic variables that explained *P. splendens* habitat distribution were "Minimum Temperature of Coldest Month" and "Isothermality", accounting for 62.7% and 22.4% of the predicted distribution respectively (Table 1). A performance test for the predicted distribution of *P. splendens* showed that the model had a good predicting performance, with a success of 86% ($p < 0.001$) for the MSS approach, and 76% ($p < 0.001$) for the 10PL approach. The area for *P. splendens* predicted by the model using both of these techniques was mostly restricted to coastal plains, ravines and valleys within its historical range. Even though there was a 33.26% difference in the total predicted suitable habitat (Table 3), both techniques identified three main areas that concentrated the large part of suitable habitat. The largest of these areas was located in the central part of the predicted distribution, an intermediate area was located in the southern range, and the smallest was in the northern section of the distribution (Fig. 2).

P. splendens potential habitat under climate change

Results from the predicted distribution under climate change scenario show that the latitudinal (north-south) range for *P. splendens* is not expected to change much in relation to the predicted distribution under current conditions (Fig 2). However, there is a large effect on the longitudinal extension of populations, with future populations not only located

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339 in the coastal range, but also spreading further east through the valleys towards the Chilean
340 central valleys. This increase in distribution also generates an important reduction in the
341 differences between the total potential areas predicted by the two aforementioned
342 techniques when compared to the predicted total area under current climatic conditions
343 (Table 3).
344
345 *Estimation of land-use change effects on P. splendens potential distribution*
346
347 When areas subjected to land-use change are subtracted from maps of potential distribution
348 a clear sign of habitat loss and fragmentation appears (Fig. 2). The largest extent of this
349 habitat loss was located in the coastal range of *P. splendens*' potential distribution and was
350 more noticeable in the central and southern range of its distribution. The effect of habitat
351 loss on potential distribution under current climatic conditions was larger in absolute values
352 (ha) in the 10PL approach in comparison to the MSS (Table 3). However, in relative terms
353 the percentage of habitat loss was practically the same, with nearly 30% of the potential
354 area of distribution being lost by anthropogenic land-use change.
355
356 In the case of projected distribution under future climatic conditions there was a
357 considerable increase of land-use change effects in absolute values in comparison to the
358 same factor under current conditions. However, surprisingly when the effects of land use
359 change were analyzed in relative terms, proportion lost values were not different for current
360 and future scenarios, and were slightly higher than 30% for both future threshold
361 approaches (Table 3).
362

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363 *Genetic diversity of P. splendens*

364

365 We obtained a total of 157 polymorphic loci with fragments ranging between 260 and 2500
366 base pairs depending on the used primer. The level of genetic diversity was similar between
367 individuals from the three sampled areas (hereafter populations). The level of
368 polymorphism varied between 98 to 100% for the studied loci. Heterozygosity and
369 Shannon's diversity index ranged between 0.21-0.24 and 0.35-0.40 respectively.

370

371 Results from the AMOVA analysis indicated that 97% of the genetic diversity was
372 distributed within populations, and only a small proportion of genetic diversity could be
373 assigned to differences between populations (ϕst= 3%, p=0.01). In addition, the Principal
374 Component Analysis (PCA) did not show any grouping patterns that may reveal presence
375 of genetic differences between sampled populations (see supplementary data).

376

377 The Bayesian Clustering analysis performed through the Structure Software using both
378 Evanno et al. (2005) and Pritchard et al. (2000) criterion resulted in a *k* number of 3 clusters
379 (Figure 3). The resulting clusters did not show a genetic structure aligned with the spatial
380 distribution of sampled populations. The first cluster included 23 individuals (15 from
381 "Palo Colorado (PC)", 6 from "Pichidangui-Los Molles (PM)", and 2 from the "Caleta
382 Quintay (CQ)" population). The second cluster was comprised of 19 individuals (4 from
383 PC, 7 from PM, and 8 from the CQ population). The third cluster included only nine
384 individuals, all of them from the PM population. The remaining 70 individuals (58% of
385 sampled individuals) showed *Q* values lower than 0.8, and therefore considered as a
386 genetically mixed group.

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387

388 *Assessment of the conservation status of P. splendens according to IUCN criteria*

389

390 Results from our study and available information in the literature allow us to evaluate this
391 species for only three out of the five criteria listed by the IUCN (see method section). We
392 could not evaluate criteria (a) because the lack of data about regeneration length. We could
393 not evaluate criteria (e) because we did not have enough data to perform extinction risk
394 analysis such as PVA. Therefore we only assessed *P. splendens* conservation status by
395 evaluating criteria (b), (c), and (d) (Table 4). To perform the analysis we used the estimated
396 habitat loss presented in Table 3 and the rough estimation of the number of individuals
397 present in the validated sites (n = 1650, see supplementary material). We complemented
398 this data with our genetic diversity analyses that suggested that the populations sampled for
399 the analysis were part of one original metapopulation now fragmented. Also, autoecology
400 of the species was considered in the assessment of the level of threat, such as lack of
401 regeneration and seed dispersion constraints (i.e., lack of seed dispersers). In addition, we
402 incorporated references that mentioned threats to *P. splendens* and its habitat (i.e. fires).
403 Moreover, we estimated habitat loss in the next decades in the area of occurrence of *P.*
404 *splendens* because the probable development of an urbanization project that potentially will
405 destroy 1700 ha of this species habitat. The results of the threat assessment are summarized
406 in Table 4. Based on our results we recommend classifying *P. splendens* as an Endangered
407 (EN) species.

408

409 **Discussion**

410

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411 This is the first attempt to study the distribution of *P. splendens* throughout its current and
412 historical range. Furthermore, this study could be considered as one of the first efforts to
413 generate landscape-level knowledge of this species, and the first aiming to provide
414 evidence to evaluate the hypothesis of an original large and continuous population that has
415 been severely reduced and fragmented by historical land-use change. In addition, it is
416 probably the first effort to assess the conservation status of this species by relying on
417 quantitative data.
418
419 Increase the knowledge about understudied taxa with potential conservation problems is a
420 key task for evaluating the real urgency to take conservation actions in the short-term
421 (McKinney 1999). However, this implies several challenges due to the scarcity of available
422 and/or reliable background data. One of the primary challenges we faced in developing this
423 study was the difficulty in finding individuals at locations where historical records show its
424 presence. From a total of 22 historical recorded presence points, we could only validate the
425 presence of *P. splendens* in eight sites. Some of the historic records are very old (e.g., La
426 Palma 1926), so we expected that some populations could have gone extinct or have
427 declined in density and extent. Therefore, it is probable that present densities were strongly
428 reduced in some sites, which could preclude us to detect the individuals that were reported
429 in the records. Furthermore, because the nature of the available records (i.e., historical
430 herbarium lists), some locations were not properly georeferenced and were described
431 simply as a locality or geographic landmark (e.g., “Chivato’s ravine”). This probably
432 decreased our success rates of finding individuals representing the populations listed in the
433 historical records. However, we also visited several points not described in the literature
434 that we thought would be suitable for the presence of *P. splendens*, with no individuals

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435 found except from one site. The difficulty of finding populations of this species within their
436 historical range of distribution support the urgent call for conserving remaining populations
437 made by Hechenleitner et al. (2005).
438
439 *Current and future suitable habitats*
440
441 The small number of presence records for *P. splendens* was another challenge we had to
442 deal in our study. Although Maxent has been reported to perform well even with small
443 number of samples (Pearson, 2007), we took additional actions by tuning the model default
444 setting to optimal levels as this procedure has shown to considerably increase the model
445 performance when dealing with small numbers of presence data (Shcheglovitova &
446 Anderson 2013).
447
448 The modelled potential original habitat distribution of *P. splendens* is concentrated in
449 coastal areas between the village of Huentelauquén and the beach resort of Algarrobo,
450 which is in accordance with the historical distribution described by other authors (e.g.,
451 Hechenleitner et al. 2005; Muñoz & Serra 2006; Sotes et al. 2013). However, the model
452 also generated a small isolated strip of suitable habitat north of Huentelauquén, for which
453 we do not have registers of known populations. Absence of registries for *P. splendens* in
454 this northern area could be just due to a lack of information because it corresponds to a
455 coastal plain far from towns and roads, and of difficult access. However, the area is also
456 coincident with the presence of the Fray Jorge National Park (30°39’50’’S; 71°40’50’’O),
457 for which there are detailed flora inventories (see Squeo et al. 2004), but no registries of *P.*

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458 *splendens*. Therefore, our result from the niche modelling needs to be carefully interpreted,
459 because the lack of other potential important variables in our model (e.g., topography, soil,
460 dispersion barriers) may result in an overestimation of suitable habitats.
461
462 Results from MaxEnt modelling using the climatic change scenario showed that *P.*
463 *splendens* would spread towards the inland areas of Central Chile, considerably expanding
464 its distribution range in comparison with its distribution under current climate conditions.
465 This trend could be explained because the increase of temperatures between 2-3°C that is
466 projected to occur in Central Chile due to climate change (CONAMA 2006; Nuñez et al.
467 2009). The *Pouteria* genus have a tropical original (Silva et al. 2009) and *P. splendens* is
468 considered a relict species from an ancient warmer climate (Gajardo 1994; Francois 2004).
469 Therefore, as temperature of the coldest month is the most important variable for this
470 species, climate change may increase this temperature in inland territories, increasing
471 potential suitable areas for this species in the future.
472
473 However, the projected potential distribution under climate change may overestimate the
474 suitable areas for *P. splendens* because our model did not account for the effect of the
475 marine fog commonly present in these coastal areas. Marine fog may have an important
476 influence in the viability of *P. splendens* populations since it provides a relatively constant
477 source of water (Hechenleitner et al. 2005; Muñoz & Serra 2006; Henríquez et al. 2012).
478 Furthermore, projected climate change it supposed to reduce precipitation in inland areas of
479 Central Chile (CONAMA 2006; Nuñez et al. 2009), which may reduce the germination
480 rates of this species (Sotes et al. 2013). Therefore, we expect that the suitable areas will

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481 increase under a climate change scenario, but probably in a magnitude much smaller that is
482 shown by the model.
483
484 In terms of the effects of land-use change in *P. splendens* distribution, our results show that
485 the current potential habitat distribution is at least 30% smaller than the original potential
486 distribution. This was regardless of the threshold used to estimate the species distribution.
487 This percentage of potential habitat loss appears not to be so substantial if we take into
488 account that this land-use change started more than 200 years ago, and that today nearly
489 one million people live within this area. However, maps show that land-use change has also
490 resulted in high levels of habitat fragmentation, which together with habitat loss, are
491 considered as the main causes of biodiversity loss in Mediterranean habitats (Soulé et al.
492 1992; Vogiatzakis et al. 2006; Cooper et al. 2012).
493
494 Fragmentation of the original habitats imposes new conditions to the remaining
495 communities, increasing edge effect and reducing the core area suitable for species (Fahrig
496 2003; Ewers & Didham 2006; Ewers et al. 2007). Furthermore, in central Chile
497 fragmentation can increase the frequency of disturbances by fires (Montenegro et al. 2004;
498 Fernández et al. 2010). Therefore, even though the modelled potential distribution suggests
499 that nearly 70% of the areas suitable for *P. splendens* still are considered as remaining
500 natural habitats, the additional effect of fragmentation may have caused an extreme
501 reduction of the original populations. These current levels of habitat fragmentation also
502 suggest that even assuming that under the future climate scenario suitable habitats for *P.*
503 *splendens* will increase; barriers to dispersion may restrict the colonization processes
504 (Engler & Guisan 2009; Henríquez et al. 2012).

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505 *Genetic diversity*

506

507 The analysis of inter simple sequence repeat (ISSR) diversity revealed that remaining *P.*
508 *splendens* populations still maintain a high level of intra-population genetic diversity. The
509 genetic variability values for *P. splendens* were similar to those reported by Nybom (2004)
510 for plants species using dominant molecular markers, and are consistent with values
511 observed for other perennial-endemic-alogamous-animal-dispersed species (Nybom 2004).
512 In relation to the inter-population genetic diversity, the low levels of genetic differentiation
513 between populations detected by AMOVA ($\phi_{ST} = 0.03$) are notably lower than those
514 observed for other plants species using dominant markers (Nybom, 2004), and also for
515 populations of other natives Chilean tree species such as *Araucaria araucana* ($\phi_{ST} = 0.13$;
516 Bekessy et al. 2002) and *Pilgerodendron uviferum* ($\phi_{ST} = 0.19$; Allnutt et al. 2003). The
517 large intra and small inter-population genetic variability was also supported by the results
518 from the Bayesian model-based clustering analysis. The analysis suggests the presence of
519 three main clusters; however only 42% of the total analyzed individuals were assigned to a
520 cluster, whereas the remaining 58% did not present enough genetic differences to be
521 considered as part of any of the three clusters, and therefore were considered a mixed
522 group.

523

524 Naturally rare or narrowly distributed plant species generally maintain lower within and
525 higher between population genetic diversity than widespread species (Gitzendanner & Solis
526 2000; Aguilar 2008); however this was not the case for *P. splendens*. Among the main
527 factors explaining a high genetic diversity in rare plant species are the recent reduction and
528 isolation of populations, or the presence of specific mechanisms that promote gene flow

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529 between populations, such as animal or wind mediated outcrossing pollination and seed
530 dispersal (Luan et al. 2006; Millar et al., 2014). In the case of *P. splendens* there is no
531 available information about the pollination mechanism, but observation from other
532 *Pouteria* species report self-pollination and insect-mediated pollination (Jordan 1996).
533 None of these mechanisms could explain the genetic diversity patterns found within and
534 between *P. splendens* populations. In relation to seed dispersal, the fruit size (2-3 cm in
535 diameter, Hechenleitner et al. 2005) suggests that seed dispersal of *P. splendens* is probably
536 mediated by large animals (Sotes et al. 2013). However, the long extirpation of large
537 animals from the actual range of *P. splendens* distribution make improbable that this
538 mechanism could be related to the genetic diversity of current populations.
539
540 Therefore, the high level of genetic variability within populations, the admixture patterns,
541 and the weak genetic structure between populations, suggest that current *P. splendens*
542 populations might be the result of a recent process of loss and habitat fragmentation. As
543 time since fragmentation is a key factor related to the loss of genetic diversity in isolated
544 populations (Aguilar et al. 2008), we may expect to see a decrease in intra and an increase
545 in inter population genetic diversity of *P. splendens* in the future, and therefore a potentially
546 increase of vulnerability to extinction of remaining populations.
547
548 *Conservation status*
549
550 Our assessment of the level of threat of *P. splendens* using the IUCN criteria revealed that
551 based on our data, this species should be reclassified as Endangered. Our results differ from
552 Hechenleitner et al. (2005) who classified *P. splendens* as critically endangered (B1ab(iii)).

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553 Unfortunately, we cannot compare our results to Hechenleitner et al. (2005) as they did not
554 report what information they used to perform their assessment.
555
556 We could not calculate or find reliable data to estimate the area of occupancy of the
557 species. Although there is data from the national forest inventory that could help to estimate
558 an area of occupancy, it seems that the inventory did not include accurate data at the
559 species level, and only three sites containing *P. splendens* were apparently mapped,
560 representing an extension no larger than 400 ha. In contrast, based in our field survey we
561 presume that the area of occupancy is not smaller than 2.000 ha. However, even taking an
562 optimist approach of 3.000 ha for the area of occupancy, this would not change *P.*
563 *splendens* to any other conservation status (e.g. from endangered to vulnerable) because
564 other assessment criteria will prevail.
565
566 We had a similar challenge for estimating the size of the remaining populations. We only
567 could calculate densities of *P. splendens* based in roughly estimation performed in each of
568 the visited sites. Thus, these estimations corresponded to projected abundance for the
569 specific visited site, but do not reflect an estimation for the entire population at that point,
570 and therefore they cannot be used to calculate the total number of individuals present in the
571 range of distribution. However, as happened with the area of occupancy variable, the
572 classification of *P. splendens* as endangered is not based in the lower number of individuals
573 we were able to estimate, but in other assessment criteria. Therefore, even though further
574 studies are able to estimate larger population's size, the threat level of *P. splendens* would
575 not change.
576

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Conclusion

Results from our work provide new and essential information for assessing current conservation status of *P. splendens* populations. The integration of our results (i.e., niche modelling, land-use change estimation, and genetic variability analysis) suggest that *P. splendens* had a large continuous population that extended through the entire range of historical recorded populations, providing essential evidence to support the continuous population assumption made previously by other authors (e.g., Hechenleitner et al. 2005; Muñoz & Serra 2006).

We were also able to update information regarding the current populations, establishing that there are only two main areas with *P. splendens* populations left, as was also suggested by previous studies. However, we found that these remaining populations are more reduced in extension and density that was previously thought. Moreover, the larger and better conserved of these populations (i.e., Pichidangui-Los Molles) is currently heavily threatened by anthropic pressure, including goat grazing, exotic tree plantations, illegal trash dumping, increasing fires, and real-estate developments. Although this area has been declared as an Urgent Priority Site for Conservation by the Chilean government (CONAMA-PNUD 2005), until now, there has not been any official conservation action taken. More concerning is the fact that currently the local government is soon to officialize a new urban development normative, which is proposing to transform more than 1700 ha of the Los Molles-Pichidangui *P. Splendens* population habitat, from natural lands, to residential areas (Seremi Medio Ambiente Valparaíso 2014). Because this probable scenario, *P. splendens* may soon change from endangered to critically endangered.

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601

602 Based on the results from our work we recommend: (1) update the information available to
603 the IUCN and evaluate the reclassification of *P. splendens* as Endangered, (2) take urgent
604 public and private actions to conserve the remaining populations of *P. splendens*; especially
605 the larger populations which is now threatened by the upcoming change in the land use
606 normative, and (3) develop more studies to fill the knowledge gap regarding this species.

607

608 **Competing interests**

609 The authors declare that they have no competing interests.

610

611 **Authors' contributions**

612 NM and IF conceived the study objectives, design and methodologies, and were in charge
613 to draft the manuscript and coordinate inputs from the other co-authors. NM performed the
614 niche modelling analysis. IF performed the land-use change analysis and mapping. BC
615 carried out the genetic analysis and helped with drafting later version of the manuscript. CO
616 helped with the literature review and in drafting the manuscript. All the above authors have
617 read the manuscript and approved this final version.

618

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628 (CONAF).

629

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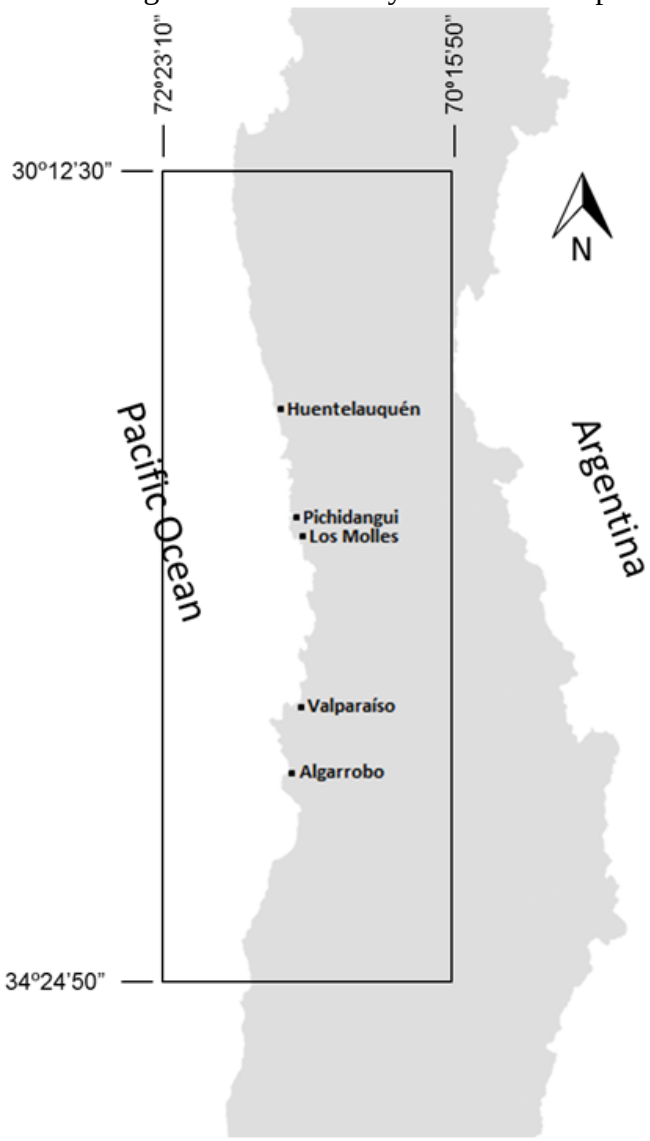
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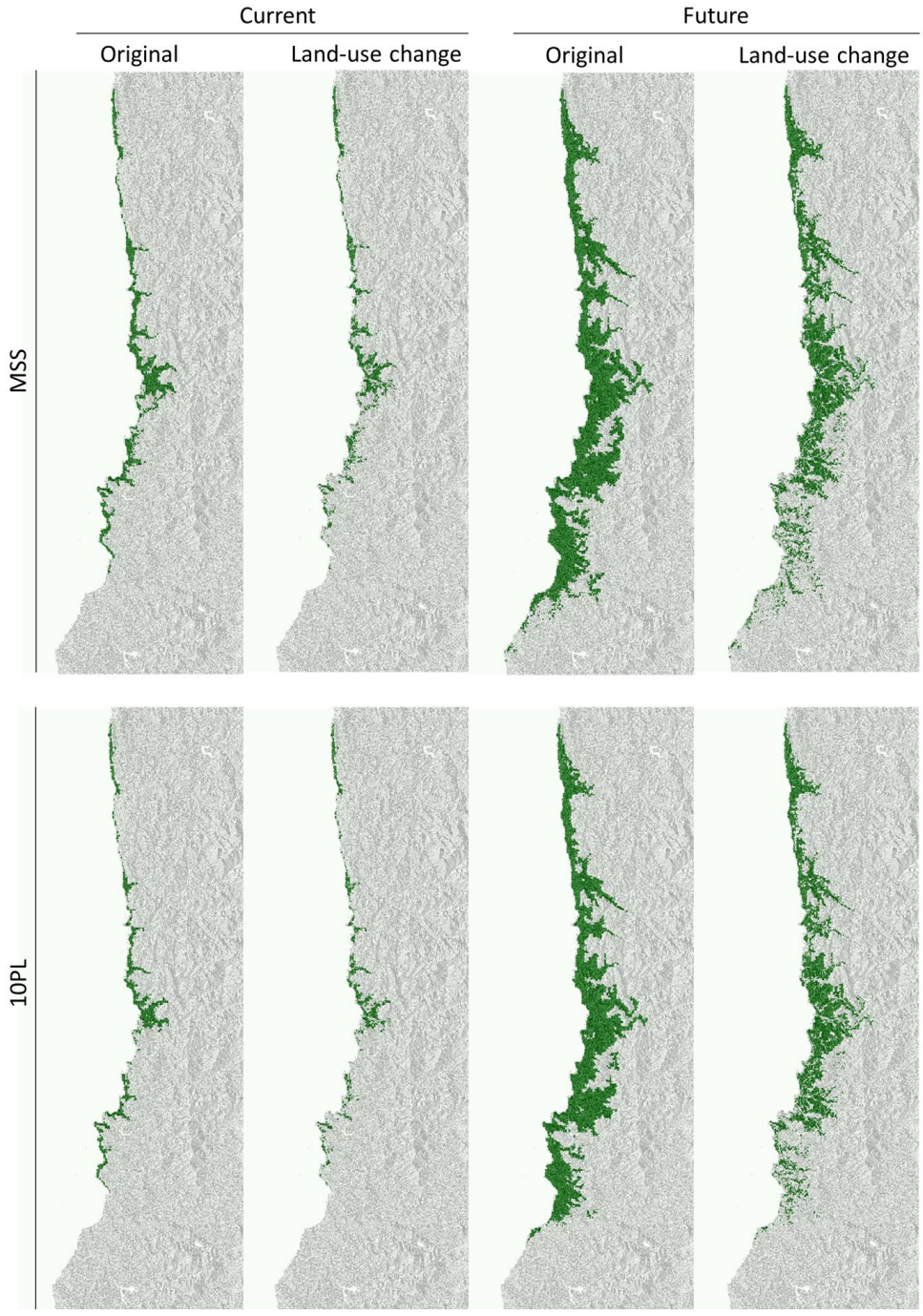
Figures

Figure 1. Area of Study. The area of study covers the coastal areas of central Chile. Historical presence records for *P. splendens* goes from the coastal village of Huentulauquén to the beach resort of Algarrobo. Dots respresent recorded presence for the species, filled dots are those for which we found in-site current presence of individuals. The black rectangle indicates the area magnified in the binary distibution maps shown in Fig. 2.



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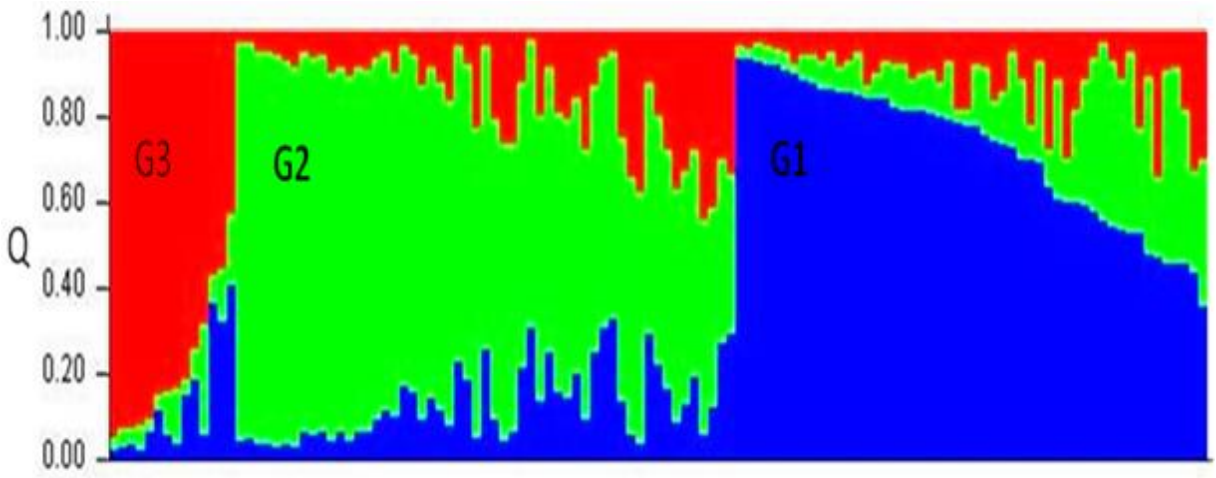
819 Figure 2. Modelled distribution maps. Maps show potential habitat (green) of *P. splendens*
820 with or without land-use change under current climate (year 1950 to 2000) and future
821 climate change scenario (year 2041 to 2060). Two different thresholds were used two
822 generate the binary maps; maximum sensitivity plus specificity (MSS) and 10 percentile
823 training presence (10PL).



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826 Figure 3. Bar plot of *Q* values. Genetic diversity analysis by using the structure software
827 shows three clusters. Blue cluster (G1) included 23 individuals; green cluster (G2) included
828 19 individuals, and red cluster (G3) contained 9 individuals. 70 individuals of *P. splendens*
829 did not pertain to any of the three clusters (*Q* values <0.8).
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833 **Tables**

834 Table 1. Bioclimatic variables from WorldClim database. The variables in bold type were
835 chosen to model the distribution of *P. splendens* after Pearson correlation analysis. The
836 most relevant variables explaining the current distribution of *P. splendens* are shown in the
837 right column.
838

Variable	Model contribution (%)
Annual Mean Temperature (Bio 1)	-
Mean Diurnal Range (Bio 2)	-
Isothermality (Bio 3)	**22.4**
Temperature Seasonality (Bio 4)	**14.4**
Max Temperature of Warmest Month (Bio 5)	-
Minimum Temperature of Coldest Month (Bio 6)	**62.7**
Temperature Annual Range (Bio 7)	**0.3**
Mean Temperature of Wettest Quarter (Bio 8)	-
Mean Temperature of Driest Quarter (Bio 9)	-
Mean Temperature of Warmest Quarter (Bio 10)	-
Mean Temperature of Coldest Quarter (Bio 11)	-
Annual Precipitation (Bio 12)	**<0.1**
Precipitation of Wettest Month (Bio 13)	-
Precipitation of Driest Month (Bio 14)	**0.1**
Precipitation Seasonality (Bio 15)	-
Precipitation of Wettest Quarter (Bio 16)	-
Precipitation of Driest Quarter (Bio 17)	-
Precipitation of Warmest Quarter (Bio 18)	**<0.1**
Precipitation of Coldest Quarter (Bio 19)	**<0.1**

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841 Table 2. Genetic variability parameters obtained from 147 bands in 121 individuals of *P.*
842 *splendens.* N: number of individuals sampled per area; I: Shannon's index; He: expected
843 heterozygosity; P (%): percentage of polymorphic loci.
844

Area	N	I	He	P (%)
Palo Colorado (PC)	48	0.40± 0.01	0.24± 0.01	100
Pichidangui-Los Molles (PM)	43	0.35± 0.01	0.21 ±0.01	99.32
Caleta Quintay (CQ)	30	0.35± 0.01	0.21 ±0.01	98.64

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852 Table 3. Potential habitat, land-use change, and habitat loss of *P. splendens* under current
853 climatic conditions (year 1950 to 2000) and future climate change scenario (year 2041 to
854 2060) with different thresholds; maximum sensitivity plus specificity (MSS) and 10
855 percentile training presence (10PL).
856

Threshold	Current			Future		
	Original (ha)	Land-use change (ha)	Habitat lost (%)	Original (ha)	Land-use change (ha)	Habitat lost (%)
MSS	140,267	99,003	29.42	614,529	420,280	31.61
10PL	93,617	65,238	30.31	521,533	362,364	30.52
Difference (%)	33.26	34.10		15.13	13.78	

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863 Table 4. IUCN Red List criteria that apply to *P. splendens* with description of the information used in the assessment and its
864 applicability in the present study. Abbreviations within the table; EOO: Extent of Occurrence, AOO: Area of Occupancy, n: mature
865 individuals (Based on Cardoso et al. 2011).

Criterion	Subcategory	Information used	Justification/decision
A. Population reduction (over 10 years or three generations)	N/A	None	Insufficient data
B. Geographic range	B1 (EOO <5000 km2 OR AOO <500 km2) AND two of: (a) fragmentation and/or locations ≤5; (b) continuing decline; (c) extreme fluctuations	1. Estimation of habitat lost 2. Genetic diversity analysis 3. Only two locations left 4. Estimation of future declination by future habitat lost and low natural regeneration (Henriquez et al 2012)	Endangered B1ab(i,ii,iii,iv,v)
C. Small population size and decline	C2 n < 10,000 AND (reduction ≥10% over 10 years or three generations OR (reduction AND (larger subpopulation ≤1000 OR 100% individuals in a single subpopulation OR extreme fluctuations)))	Estimation of the number of individuals present in validated locations	Vulnerable C2a(i)
D. Very small or restricted population	D2 n < 1000 OR AOO <20 km2 OR locations ≤5	Confirmation *in situ*, estimations of habitat lost and literature (Hechenleitner 2005, Henriquez et al 2012)	Vulnerable D2
E. Quantitative analysis of extinction risk	N/A	None	Insufficient data

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866 **Supplementary material**

867

868 Supplementary material 1. *Pouteria splendens* population presence records information.
869 Sites are listed from north-to-south. Sites in bold type represent those for which we were
870 able to find individuals. Approximate numbers of individuals are only presented for
871 populations found during fieldwork. Numbers are based in visual estimations from
872 fieldwork and recent satellite images. [a]This site was not in the historical records and was
873 registered during the field work. [b]This was considered as an spatial outlier point and was
874 not used in the modeling process because corresponded to a registry from 1926 for which
875 we did not have enough reliance.

Site name	Coordinates		Approx. number of individuals	Herbarium
	Latitude	Longitude		
Quebrada el Buitre	31°26'52"S	71°35'3"W		CONC
Norte de Los Vilos	31°41'16"S	71°31'45"W		CONC
La Totora	31°54'30"S	71°27'41"W		CONC
Estación Ingeniero Barriga	32°1'57"S	71°29'54"W		CONC
Sur de Los Vilos	32°3'20"S	71°30'54"W		CONC
Entre Pichidangui-Los Vilos	32°3'53"S	71°31'4"W		CONC
Fundo Palo Colorado	32°4'17"S	71°29'24"W	60	CONC
Fundo Palo Colorado Oeste[a]	32°5'34"S	71°30'55"W	50	a
Silla del Gobernador	32°7'50"S	71°28'52"W		SGO
Pichidangui	32°8'53"S	71°31'38"W	300	CONC
Los Ermitaños	32°11'0"S	71°31'46"W	500	CONC
Kilómetro 180 ruta 5 norte	32°12'35"S	71°30'41"W	200	CONC
Los Molles	32°14'9"S	71°31'18"W	400	CONC
Quebrada el Chivato	32°13'59"S	71°29'33"W	50	CONC
Quebrada Las Docas	33°2'16"S	71°39'17"W		CONC
Las Lúcumas	33°2'22"S	71°38'56"W		CONC
Valle del Agua Potable	33°2'33"S	71°38'42"W		CONC
La Palma[b]	32°58'32"S	71°16'31"W		SGO
Curauma-Las Docas	33°4'27"S	71°39'35"W		CONC
Laguna Verde	33°6'15"S	71°40'17"W		CONC
Curaumilla	33°8'8"S	71°42'21"W	20	CONC
Caleta Quintay	33°12'18"S	71°41'53"W	70	SGO
Algarrobo	33°22'53"S	71°40'51"W		CONC

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Supplementary material 2. Corrected Akaike information criterion (AICc) for the best 10 combinations of features and regularization multiplier values of MaxEnt (e.g LQ 1 = linear quadratic and regularization multiplier equal to 1).* Auto features corresponds to using MaxEnt with the default configuration parameters for small samples.

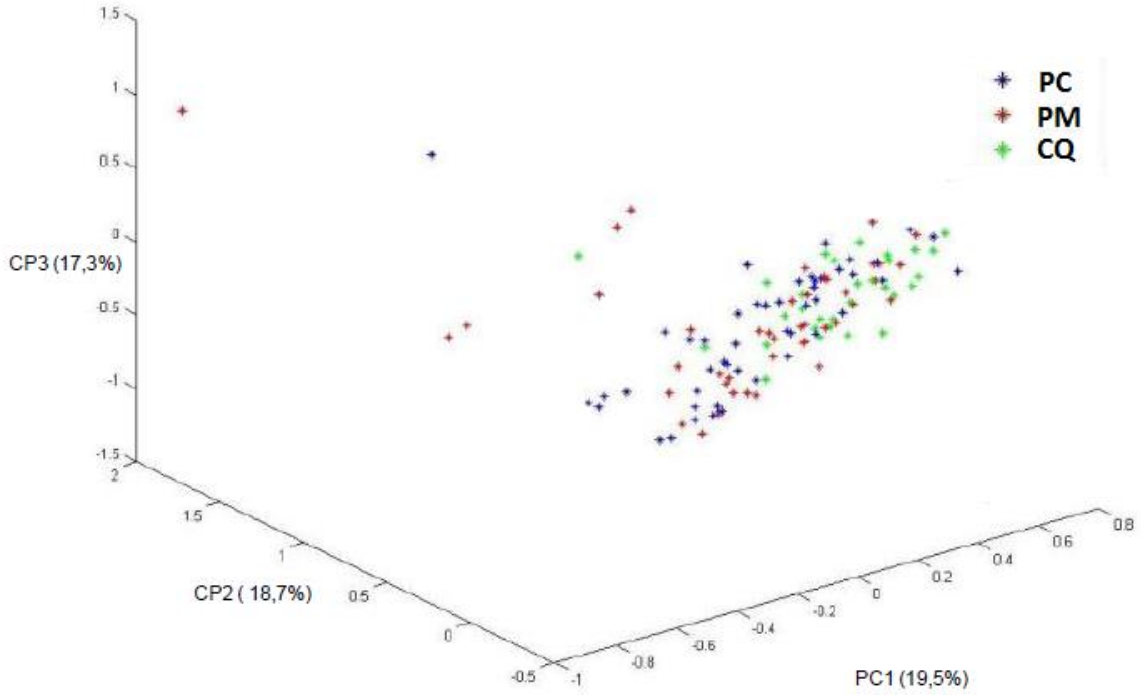
Combination of features	AICc score
LQ 1	421.17
L 1	428.23
LQ 2	429.08
L 2	441.58
LQ 5	447.04
LQH 5	455.14
Auto features*	457.90
LQH 1	457.90
LQH 2	465.54
H 5	468.05

Supplementary material 3. ISSR primers used for the analysis of *Pouteria splendens* . R can be either A or G nucleotide, whereas Y can be either C or T nucleotide

Primer	Sequence	Annealing temp (C)	Band size range (base pairs)
825	ACA CAC ACA CAC ACA CT	58	500-2700
844	CTC TCT CTC TCT CTC TRC	54	420-1900
849	GTG TGT GTG TGT GTG TYA	57	410-1700
854	TCT CTC TCT CTC TCT CRG	54	430-2080
856	ACA CAC ACA CAC ACA CYA	59	430-2080

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898 Supplementary material 4. Principal Component Analysis for 121 individuals of *P.*
899 *splendens* and 147 ISSR loci. Sampled populations were PC (Palo Colorado), PM
900 (Pichidangui-Los Molles), CQ (Caleta Quintay).
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