Plant-insect interactions patterns in three European paleoforests of the late-Neogene – early-Quaternary

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Plants and insects are constantly interacting in complex ways through forest communities since hundreds of millions of years. Those interactions are often related to variations in the climate. Climate change, due to Human activities, may have disturbed these relationships in modern ecosystems. Fossil leaf assemblages are thus good opportunities to survey responses of plant-insect interactions to climate variations over the time. The goal of this study is to discuss the possible causes of the differences of plant-insect interactions' patterns in European paleoforests from the Neogene – Quaternary transition. This was accomplished through three fossil leaf assemblages: Willershausen, Berga (both from the late Neogene of Germany) and Bernasso (from the early Quaternary of France). In Willershausen it has been measured that half of the leaves presented insect interactions, 35% of the fossil leaves were impacted by insects in Bernasso and only 25% in Berga. The largest proportion of these interactions in Bernasso were categorized as specialist (mainly due to galling) while in Willershausen and Berga those ones were significantly more generalist. Contrary to previous studies, this study did not support the hypothesis that the mean annual precipitation (MAP) and temperature (MAT) were the main factors that impacted the different plant-insect interactions' patterns. However, for the first time, our results tend to support that the hydric seasonality and the mean temperature of the coolest months could be potential factors influencing fossil plant-insect interactions.

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ABSTRACT

Plants and insects are constantly interacting in complex ways through forest communities since hundreds of millions of years. Those interactions are often related to variations in the climate. Climate change, due to Human activities, may have disturbed these relationships in modern ecosystems. Fossil leaf assemblages are thus good opportunities to survey responses of plant-insect interactions to climate variations over the time. The goal of this study is to discuss the possible causes of the differences of plant-insect interactions' patterns in European paleoforests from the Neogene – Quaternary transition. This was accomplished through three fossil leaf assemblages: Willershausen, Berga (both from the late Neogene of Germany) and Bernasso (from the early Quaternary of France). In Willershausen it has been measured that half of the leaves presented insect interactions, 35% of the fossil leaves were impacted by insects in Bernasso and only 25% in Berga. The largest proportion of these interactions in Bernasso were categorized as specialist (mainly due to galling) while in Willershausen and Berga those ones were significantly more generalist. Contrary to previous studies, this study did not support the hypothesis that the mean annual precipitation (MAP) and temperature (MAT) were the main factors that impacted the different plant-insect interactions' patterns. However, for the first time, our results tend to support that the hydric seasonality and the mean temperature of the coolest months could be potential factors influencing fossil plant-insect interactions.

INTRODUCTION

Climate is a major factor affecting the extension, structure, and composition of terrestrial ecosystems (Taylor et al., 2012; Frank et al., 2015). Hence, past climatic oscillations are of special importance for understanding and interpreting biotic changes in the past (e.g., DeChaine & Martin, 2006) and are of interest in terms of forecasting the biotic response to future global warming (Meehl et al., 2007). Nowadays, it is clear that Human activities have now reached a global impact affecting components of the Earth system as a whole (Turner et al., 1990; Heller & Zavaleta, 2009). In terrestrial ecosystems, arthropods are one of the most important components in biodiversity (Yang & Gratton, 2014) and their interactions with plants are essential for terrestrial food webs (Forister et al., 2015). Many modern ecological studies are focusing on these interactions between plants and insects but interpretations may be limited, therefore a combination with studies focused on the fossil record is necessary (Wilf, 2008). Studies on fossil insect herbivory have provided a variety of ecological and evolutionary information over long periods of time, such as climate (e.g., Wappler, 2010; Wappler et al., 2012), the evolutionary impact of plant radiations (e.g.,Labandeira, 2012; Labandeira & Currano, 2013), food web dynamics (e.g., Wappler & Grímsson, 2016), extinction patterns (e.g., Labandeira, 2002; Labandeira, Johnson & Wilf, 2002; Donovan et al., 2016), and ecosystem recovery after extinction events (e.g., Wappler et al., 2009; Labandeira, Kustatscher & Wappler, 2016). They have also shown that biodiversity loss may greatly impede trophic interactions and change the overall food web structure of ecological systems (Haddad et al., 2009). Moreover, there is increasing concern about the loss of biological diversity from ecosystems (Hooper et al., 2012).

The large amount of Plio–Pleistocene fossil records offers an exceptional possibility to estimate the evolution and dynamics of associations between plant species and their dependent insect-herbivore species, as descriptions of Plio–Pleistocene floral changes by combining different and complementary data (Tzedakis, Hooghiemstra & Pälike, 2006; Médail & Diadema, 2009; Postigo Mijarra et al., 2009; Magri, 2010; Migliore et al., 2012). Although a few isolated records of specialized phytophagy categories have been reported from the Pliocene (Straus, 1977; Givulescu, 1984; Titchener, 1999), only a single systematic survey of plant–arthropod interactions has been carried out on an early Pleistocene flora (Adroit et al., 2016).

Thus, an ideal setting for the evaluations of relationships among global climate and biodiversity under conditions warmer than today, but with a similar paleogeographic configuration (Raymo *et*

67 *al*. 2011; Rohling *et al*. 2009) is possible throughout the famous upper Pliocene fossil

68 Lagerstätten Willershausen (3.2 – 2.6 Ma; MN 16/17) (Hilgen, 1991; Mai, 1995) and the

69 comparisons with Berga (Germany, late Pliocene) and the French Pleistocene locality of

70 Bernasso (Adroit et al., 2016). Willershausen and Berga outcrops are of similar age (Piacenzian)

71 and are located in the surroundings of the Harz Mountains, Germany (Figure 1). The

72 Willershausen paleoforest was dominated by typical taxa of hilly mesophytic woodland

73 (Ferguson & Knobloch, 1998; Knobloch, 1998) such as *Acer, Aesculus, Carpinus, Fagus,*

74 *Quercus, Sassafras, Tilia* (Mai, 1995; Knobloch, 1998) and other taxa such as *Parrotia, Zelkova*

75 and *Liquidambar* were also characteristic elements of Willershausen (Mai, 1995). All of these

76 taxa were also found in Berga (Mai & Walther, 1988). The presence of these taxa indicates

77 relatively warmer conditions in Europe than today during the late Pliocene (Uhl et al., 2007;

78 Thiel, Klotz & Uhl, 2012). Most plant fossil evidence from Central Europe outcrops (Haywood,

79 Sellwood & Valdes, 2000; Uhl et al., 2007; Williams et al., 2009; Thiel, Klotz & Uhl, 2012) and

80 data from marine isotopes, geological evidences (Driscoll & Haug, 1998; Haug, Tiedemann &

81 Keigwin, 2004) are directing in the same way. Bernasso is younger than the German outcrops,

82 estimated around 2.16 Ma – 1.96 Ma (Suc, 1978; Leroy & Roiron, 1996). It is located in

83 southern France, 5km far away from Lunas in the department of Hérault (Suc, 1978; Leroy &

84 Roiron, 1996; Adroit et al., 2016). The Bernasso fossil leaf assemblages is mainly dominated by

85 the genera *Carpinus*, *Parrotia*, *Acer* and *Sorbus* (Leroy & Roiron, 1996; Adroit et al., 2016)

86 wherein many plant species are in common with the German fossil leaf assemblages. Detailed

87 descriptions are available in Leroy & Roiron (1996) and Adroit et al. (2016). The decreasing

88 temperatures, from ca. 18°C to 14°C throughout the Pliocene (Thunell, 1979; Ravelo et al., 2004;

89 Hansen et al., 2013) lead to the dominant European vegetation changing gradually from highly

90 diverse subtropical and warm-temperate forests to temperate deciduous forests with East Asian

91 and partly North American affinities (Mai, 1995).

92 Through the comparison of three European forest plant communities of the Plio-Pleistocene, the

93 aim of this study was to understand how climatic parameters could have impacted plant-insect

94 interactions of fossil leaves. It has been expected that the difference of estimated mean annual

95 temperatures between those paleoforests could have a major impact on the quantity of the plant-

96 insect interactions (Coley & Aide, 1991; Zvereva & Kozlov, 2006; Currano, Labandeira & Wilf,

97 2010). Moreover, the estimated mean annual precipitations of the fossil outcrops should be

98   negatively related to the galls proportions observed on the fossil leaves (Fernandes & Martins,

99   1985; Fernandes & Price, 1988; Price et al., 1998; Lara, Fernandes & Gonçalves-Alvim, 2002).

100   Our results provide the first approach on plant-insect interactions from the Plio-/Pleistocene in

101   European paleoecosystems.

102

103   **STUDY AREA**

104   *Willershausen, Lower-Saxony, Germany*

105   Geological studies of Willershausen dated back to the end of the 19th century (e.g., Wegele,

106   1914); see details in (Wegele, 1914; Ferguson & Knobloch, 1998; Meischner, 2000). The

107   absence of bioturbation gave rise to one of the exceptionally well preserved floras and faunas

108   (Briggs et al., 1998). The Willershausen site was a lake which developed in a pond due to the

109   dissolution of underlying Permian evaporites and has been buried by Triassic and Early Jurassic

110   sediments (Briggs et al., 1998; Meischner, 2000; Kolibáč et al., 2016). Today, Willershausen is

111   an abandoned clay mining operation and it is included in the Geopark Harz, Braunschweiger

112   Land, Ostfalen since 2012. This paleolake was ca. 200m in diameter and approximately 10m

113   deep with a narrow sand beyond which the sides inclined abruptly towards the bottom of the lake

114   (Meischner, 2000). Willershausen geology has been described by (Von Koenen, 1895) and

115   detailed compilations can be found in (Vinken, 1967; Ferguson & Knobloch, 1998; Meischner,

116   2000).

117   The leaves used in this study are stored in different museum collections in Germany. The

118   majority (6546 leaves) is located at the Geoscience center of the University of Göttingen

119   (GZG.W collection). Additional fossil leaves are stored in the Staatliches Museum für

120   Naturkunde Stuttgart (SMNS.W collection, 957 leaves), in the collections of TU Clausthal of

121   Clausthal-Zellerfeld (320 leaves), in the Naturkundemuseum im Ottoneum of Kassel (NMOK.W,

122   236 leaves) and in the Senckenberg Natural History Collections Dresden (14 leaves). Some of

123   the best well-preserved fossil specimens are presented in Figure 2.A. The flora from

124   Willershausen comprised a rich vegetation community including the presence of *Acer, Alnus,*

125   *Betula, Carpinus, Carya, Fagus, Pterocarya, Populus, Quercus, Tilia, Ulmus, Zelkova* (Straus,

126   1977; Ferguson & Knobloch, 1998; Knobloch, 1998). The vertebrates *Anancus (Mastodon)*

127   *arvernensis* and *Tapirus* were found in Willershausen and seems to indicated a Piacenzian age

128   (late Pliocene, ca. 3.2 – 2.4 Ma; MN 16/17; (Mai, 1995), which is corroborated by the presence

129   of *Parrotia persica* and *Liquidambar europaeum* (Mai, 1995).

130   Most plant fossil evidence from Willershausen indicates warmer conditions than today (Ferguson

131   & Knobloch, 1998). The mean annual temperature (MAT) in Willershausen was estimated

132   between 10.6°C and 15.6°C on the base of the leaf morphology and of diversity of plant species

133   niches (Table S1; Uhl et al., 2007); these different approaches explain the wide range of

134   temperature estimated. The mean temperature of the coldest month (CMMT) is estimated

135   between 0.6°C and 3.2°C and the mean annual precipitation (MAP) between 897 and 1151 mm

136   per year (Table S1; Uhl et al., 2007; Thiel, Klotz & Uhl, 2012).

137

138   *Berga, Thüringen, Germany*

139   Berga was a lake in which compressions and impressions (some with the cuticles preserved) of

140   leaves were found in silty sediments (Mai & Walther, 1988). It is 70km far from the

141   Willershausen outcrop. The stratigraphic age of the Berga sediments is estimated on the basis of

142   sedimentological correlations referring to the Piacenzian (ca. 3 Ma – 2.6 Ma) (Bachmann et al.,

143   2008).

144   This leaf collection (534 specimens) is housed in the collection of the Senckenberg Natural

145   History Collections Dresden, Germany. It contained many fossils of different origins (Mai &

146   Walther, 1988), including 30 angiosperms leaf taxa (Figure 2.B). They represent different

147   environments: a freshwater plant community, a swamp and riparian associations and a zonal

148   mixed broadleaved conifer forest (which dominates the taphocoenosis). The temperatures were

149   estimated with the same approach as Willershausen; MAT is estimated between 7.4°C and

150   16.6°C, the CMMT is between -4.3°C to +0.6°C and the MAP is between 897 and 1297 mm per

151   year (Table S1; Uhl et al., 2007; Thiel, Klotz & Uhl, 2012).

152

153   *Bernasso, France*

154   Bernasso was a lake developed when a basaltic flows shut off a canyon valley (Leroy & Roiron,

155   1996). Diatomites were formed and fossil leaves, often with rest of cuticle, were preserved. It is

156   located close to Lunas (Hérault, Southern France) (Leroy & Roiron, 1996; Adroit et al., 2016).

157   The fossil deposit is dated from the early Pleistocene on the basis of K/Ar analysis (Ildefonse et

158   al., 1972) on a basaltic dyke that crosses the diatomite layers. A complementary analysis on

159   cyclostratigraphy (Suc & Popescu, 2005) and paleomagnetism (Ambert et al., 1990) corroborated

160   these results and estimated an age around 2.16 to 1.96 Ma.

161   The collection included 800 fossil leaves and 535 specimens well-preserved were described in

162   (Adroit et al., 2016). These same specimens were also used for comparisons in the present study.

163   The fossil leaves were conserved the Institut des Sciences de l'Evolution de Montpellier in

164   France. Different preparation of fossil leaves were done by (Leroy & Roiron, 1996) and their

165   impact on interpretation were discussed in (Adroit et al., 2016). The flora represents a

166   mesothermic forest, mixing Mediterranean and Caspian elements (Suc, 1978; Leroy & Roiron,

167   1996). The MAT in Bernasso was estimated about 14-15°C and the MAP is around 1500 mm/y

168   (Table S1; Leroy & Roiron, 1996). It is important to note that CLAMP results in Bernasso

169   suggest a possibly lower temperature around 7°C (Table S1; Girard et al. 2017, unpublished

170   data).

171

172

173   **DATA ANALYSES**

174   *Plant-insect interaction identifications*

175   The plant-insect interactions were identified following the Guide to Insect (and Other)

176   Damage Types on Compressed Plant Fossils (Labandeira et al., 2007). The damages type (DTs)

177   are easily recognizable thanks to the black reaction mark surrounding them (Labandeira, 2002;

178   Labandeira et al., 2007). They are divided in 7 Functional Feeding Groups (FFG): hole feeding,

179   margin feeding, skeletonization, surface feeding, piercing & sucking, mining and galling. Leaves

180   without damage were also categorized in an eighth FFG called the undamaged leaves. The leaves

181   undamaged has been take into account as a proxy of the non-palatability of the leaves, thus can

182   be considered as another FFG. The leaves were examined under a binocular Leica MZ95 and all

183   photographed with a Canon EOS 350D camera fitted with a Canon EF-S 60-mm f/2.8 macro

184   lens. A Nikon Coolpix E4500 was used sometimes for precise pictures through the binocular. All

185   pictures were developed using Abode Lightroom CC v.2015 especially in order to improve

186   contrast of the leaf. The insect interactions on leaves were scored according to the richness,

187   frequency and distribution on the different plant species for each outcrop. For each DT, a host

188   specificity value has been attributed by Labandeira et al. (2007) that allowed to classify our DTs

189   into generalist interactions (made by polyphagous organisms) and specialized interactions (made

190   by monophagous organisms) (Labandeira, 2002). Detailed plates of fossil leaves from

191   Willershausen are available in Table S2 including the original descriptions of the plant-insect

192   interactions made by (Straus, 1977) and our actual updates with the guide of insect (and other)

193   damage types on compressed plant fossils (Labandeira et al., 2007).

194   The results obtained for Willershausen and Berga were compared to those recently published for

195   the outcrop of Bernasso (Adroit et al., 2016). For some comparisons with Bernasso, new values

196   were calculated based on raw data.

197

198   *Statistical analyses*

199   For each outcrop, the statistical analyses were performed on two different databases as described

200   in Knor et al. (2012). The first one is the whole assemblage of plant-insect interactions. The

201   second one considers only the interactions of the species that are significantly represented (more

202   than 20 leaves). The quantitative analyses were done in R version 3.1.2 (R Development Core

203   Team, 2014). The differences among the proportions of occurrences from all FFG were tested

204   with Chi-squared-test. The remaining information needed for this test was obtained by using the

205   generalized linear model of binominal distribution. Sample-based rarefaction curves were done

206   to compare the different damage richness and the different plant richness between the outcrops

207   (Gotelli & Colwell, 2001). At last, in order to observe the distributions of plant species according

208   to the FFG among the different fossil leaf assemblages, principal component analysis (PCA)

209   were performed with the software Past3 (v3.14) (Hammer, Harper & Ryan, 2001) in a biplot.

210   PCA has been useful to assess relationships of the plant species for each FFG. The data matrices

211   used for it considered the frequency of each eight FFGs for each plant species of each outcrop

212   (i.e. for each outcrops a matrix such as FFG frequency x leaf morphotype).

213

214   **RESULTS**

215   *Comparisons of insect interactions and plant species richness.*

216   In Willershausen 50.4% of the leaves are damaged, and only 25.1% in Berga. This percentage

217   was 34.6% in Bernasso (Adroit et al., 2016). These differences are statistically significant

218   ($p<0.001$) (Figure 3, Table 1).

219   The frequencies of generalist interactions are 42.8% for Willershausen, 17.8% for Berga and

220   19.8% for Bernasso (Adroit et al., 2016). Only Willershausen frequency is significantly different

221   from the others ($p<0.001$). Willershausen leaves have especially much more hole feedings

222   (26.9%) and margin feedings (9.9%) than Berga (respectively 12.7% and 1.9%) and Bernasso

223   leaves (respectively 9.8% and 7%) (Figure 3).

224   The frequencies of specialized interactions are 11.2% for Willershausen, 8.4% for Berga and

225   17.9% for Bernasso (Adroit et al., 2016). Only the Bernasso frequency is significantly different

226   from the others ($p<0.001$). This difference is mainly due to the important quantity of galling in

227   Bernasso (12%) which is significantly higher than in Willershausen and Berga, respectively 7%

228   and 6% ($p<0.01$) (Figure 3).

229   Rarefaction tests on plant species richness highlight that Willershausen has more plant species

230   (>100) than Berga (33) and Bernasso (20) which has the less one (Figure 4). However, the DT

231   richness in Willershausen (36 DTs) and Berga (25 DTs) are lower than in Bernasso (40 DTs)

232   (Figure 4).

233

234   *Structure of the paleoforests with the damage distribution on plant species*

235   Figure 5 presents the different PCA realized for the 3 outcrops with the data of plant and DT

236   diversities. For each outcrop, only the first two axes are presented as for Willershausen they

237   represent 77% (Figure 5A), for Berga 93% (Figure 5B) and for Bernasso 91% (Figure 5C) of the

238   whole distribution.

239   For Willershausen (Figure 5A) the FFGs hole feeding and skeletonization are positively

240   correlated with PCA-axis 1 (respectively 0.76 and 0.61) and undamaged is negatively correlated

241   with this axis (-0.97) (Data S1). Skeletonization and galling are positively correlated with PCA-

242   axis 2 (respectively 0.62 and 0.63) while hole feeding is negatively correlated with this axis (-

243   0.73) (Data S1). Concerning the species, three pools of plant species can be distinguished. The

244   *Tilia* (*T. saportae, T.* cf. *saviana*), the *Ulmus* (*U. caprinifolia, U. campestris*), the *Fagus* (*F.*

245   *grandifolia, F. pliocenica*), *Acer integerrimum* and *Quercus roburoides* are all along the positive

246   part of the PCA-axis 1. The leaves of these species have the highest DT frequency of hole

247   feeding and skeletonization. A second set of taxa is composed, for the most evident species, by

248   *Acer cappadocicum, A. laetum, Carya minor*, cf. *Magnolia* sp1 and 2, *Populus willershausensis*,

249   *Quercus praeerucifolia* and *Zelkova ungeri*. They are along the negative part of the PCA-axis 1

250   and along the positive part of the PCA-axis 2. They are mainly affected by the FFG galling

251   (specialized interaction) or have no damage. At last, the third set of species is composed of

252 Fagales (*Fagus sylvatica*, all the *Quercus*, *Alnus* and *Betula* species) and is in the negative part of

253 the PCA-axis 2. These leaves are mainly undamaged or only impacted by hole feeding

254 (generalist interaction).

255 For Berga (Figure 5B) the FFGs hole feeding, skeletonization and undamaged are positively

256 associated with the PCA-axis 1, respectively with a correlation of 0.56, 0.73 and 0.99 (Data S1).

257 Hole feeding and skeletonization are also correlated with the PCA-axis 2, negatively for hole

258 feeding (-0.79) and positively for skeletonization (0.66) (Data S1). Concerning the species, we

259 can note that *Taxodium dubium, Zelkova ungeri, Cercidiphylum crenatum* and *Acer*

260 *integerrimum* are correlated with this undamaged category. *Fagus attenuata, Acer tricuspidatum*

261 and *Quercus* sp. are mainly correlated with hole feeding.

262 For Bernasso (Figure 5C; (Adroit et al., 2016), the skeletonization and galling are positively

263 correlated with the PCA-axis 1 (0.82 and 0.92) while undamaged is negatively correlated with

264 this axis (-0.94) (Data S1). Hole feeding and skeletonization are positively correlated with PCA-

265 axis 2 (0.92 and 0.25) while undamaged and galling are negatively correlated with this axis (-

266 0.25 and -0.37) (Data S1). *Acer monspessulanum* and *Sorbus domestica* are in the positive part of

267 the PCA-Axis 1 while the other are in the negative one (to note that *Parrotia persica* is close to

268 zero). Concerning the PCA-axis 2, *Acer monspessulanum, Carpinus orinetlais* and *Carya minor*

269 are in the positive part of the PCA-axis 2 while the others are in the negative part (to note that

270 *Zelkova ungeri* is close to zero).

271 Furthermore, *Zelkova ungeri* is a species found in the 3 outcrops (Figure 5) and comparing to its

272 position in the different PCAs, we can note that *Z. ungeri* is mostly associated with the FFG

273 undamaged. However, for other common plant species, their relative position on the PCAs could

274 be different. *Acer integerrimum* in Berga (Figure 5B) is mostly associated with the FFG

275 undamaged while in Willershausen is opposite to this FFG as it is mainly associated to the FFG

276 hole feeding and more weekly with skeletonization, margin and galling (Figure 5A).

277 Comparing Willershausen and Bernasso (Figure 5A & 5C), *A. monspessulanum* is mainly

278 associated to skeletonization and galling in Willershausen (Figure 5.A) and in Bernasso it is with

279 skeletonization, galling too but also with hole feeding (Figure 5C).

280 *Sorbus domestica* and *Carpinus orientalis* are both associated to the FFG undamaged in

281 Willershausen (Figure 5A). In Bernasso, *S. domestica* is associated with galling and

282 skeletonization and *C. orientalis* is associated with hole feeding and undamaged (Figure 5C).

283   *Carya minor* is associated to skeletonization and galling in Willershausen (Figure 5A) while in

284   Bernasso it is associated to undamaged and hole feeding (Figure 5C). *Parrotia persica* is

285   associated to galling and undamaged in Bernasso (Figure 5C) while in Willershausen, it is

286   associated with the FFGs skeletonization and galling (Figure 5A).

287

288   **DISCUSSION**

289   *Floristic richness and herbivory representativeness*

290   All genera and at least 22 plant species from Berga leaf assemblage are also present in the

291   Willershausen assemblage (Table S3). It can be explained by the geographical and stratigraphical

292   proximity of the two outcrops. Bernasso had nearly the same composition of plant genera found

293   in Willershausen (except *Ilex* only found in Bernasso) and also the majority of plant species

294   (Table S3) despite its geographical situation and its younger age. There is quite a difference of

295   plant richness between Bernasso and Berga, but the genera are the same (Table S3). This may

296   suggest a difference in specific richness between those paleoforests. Rarefaction data indicated

297   for the Willershausen leaf assemblage a highest plant species richness than the ones of Berga and

298   Bernasso (Figure 4). However, the original sample size is considerably larger in Willershausen

299   and could have led to artificial differences of plant species richness between the outcrops (Table

300   1). However, a bias due to the sample size is unlikely as Bernasso has the highest DT richness

301   while the plant species and the quantity of leaves are lower than the one of the Willershausen

302   assemblage.

303   Sampling effort tests indicate that enough specimens were taken into account to have a

304   representative overview of the interactions on plant species found into the different outcrops

305   (Figure 4). The large standard deviation observable on the Willershausen rarefaction curves on

306   Figure 4 is due to this size of the fossil collection that includes around 8.000 specimens while the

307   others are only 534 for Berga and 535 for Bernasso.

308

309   *Relations between herbivory and the different mean annual temperatures estimated*

310   Climatic conditions seem to be in relation with variations in richness and frequency of

311   plant-insect interactions (Currano, Labandeira & Wilf, 2010). If an increase of temperature

312   seems to stimulate insect herbivory (Coley & Aide, 1991; Coley & Barone, 1996; Zvereva &

313   Kozlov, 2006; Currano, Labandeira & Wilf, 2010), it is still difficult to understand the complete

314   role of temperature in the modulation of herbivory (DeLucia et al., 2012).

315   Thiel, Klotz & Uhl (2012) indicated, through leaf morphological analyses, that temperatures

316   estimated for Willershausen were approximately 3°C higher than those for Berga. These

317   paleoforests were geographically very close to each other (less than 70 km) and at a similar

318   latitude (51°N) (Figure 1). Today the nearest meteorological stations of these locations

319   (Willershausen: Göttingen, Lower-Saxony; Berga: Nordhausen, Thüringen) indicates the same

320   mean annual temperature also for the coldest and warmest months over the last years

321   (www.worldweatheronline.com). Such current similarities make the argument for similar

322   paleoclimates of the two fossil localities if they were strictly of the same age. However, between

323   3 Ma and 2.5 Ma, CO_2 concentration progressively decreased (Kürschner et al., 1996; van de

324   Wal et al., 2011) implicating a continuous decrease of mean annual temperatures (Willeit et al.,

325   2015). Consequently, as Willershausen was warmer than Berga, the paleoforest of Willershausen

326   grew under higher atmospheric CO_2 concentration than the Berga paleoforest. It seems to

327   corroborate by the higher damage frequency observed in Willershausen that can have been

328   favored by an increase of C/N ratios and an increase of photosynthesis rates (due to the high CO_2

329   concentration) (Bezemer & Jones, 1998; Stiling & Cornelissen, 2007; DeLucia et al., 2012).

330   However, Willershausen and Berga had different sedimentological contexts and the preservation

331   of the fossil leaves did not follow the same taphonomical constrains in the two outcrops. This

332   could have influenced interpretation of the climate through morphological analyses.

333   For this reason, Thiel, Klotz & Uhl (2012) were in favor of the Coexistence Approach for

334   climate interpretation which estimated similar temperature for Berga and Willershausen. It has

335   been highlighted that the diversity of insects is often correlated to richness of plant species

336   (Siemann, Tilman & Haarstad, 1996; Wright & Samways, 1998; Knops et al., 1999; Mulder et

337   al., 1999) and should be expected to have higher damage richness in the more diverse paleoforest

338   (Price, 1991, 2002). Thus, the higher richness and frequency of damage in Willershausen than in

339   Berga could also be due to a higher insect diversity. Nevertheless, despite its higher plant

340   richness Willershausen had less DT richness than the fossil leaf assemblage of Bernasso (Figures

341   3, 4). Bernasso had also more damage richness and frequency than Berga (Figures 3, 4). Thus,

342   these observations make this assumption unsustainable for our study. It is also conceivable that

343   the relative abundance of a plant species in those paleoforests could partly explain the herbivory

344   measured. Indeed, more a plant species is represented in the forest community, then more

345   individuals of the plant species have had a chance to be damaged by insect feeding (Feeny,

346   1976). Unfortunately, for the fossil record, it is not possible to support this assumption because

347   the leaf quantity of a plant species from an outcrop cannot be correlated to the relative abundance

348   of this plant species in the paleoforest. Consequently, the difference of plant species richness

349   observed between the outcrops could be firstly due to differences of fossil preservation than

350   more to differences among paleoecosystems.

351   For Bernasso, the latitudinal position is different from Berga and Willershausen, as it located

352   1,000 km to the South. It has been highlighted that the insect diversity increases getting closer to

353   the tropics (Hutchinson, 1959; Klopfer, 1959; Klopfer & MacArthur, 1960; MacArthur, 1972;

354   Coley & Barone, 1996; Fraser, 2017). The southern position of Bernasso could partly explain the

355   measured damage type richness. Nevertheless, the quantity of damage is not exclusively linked

356   to the insect diversity (Currano, Labandeira & Wilf, 2010). Latitudinal differences could led to a

357   difference of thermal seasonality (Saikkonen et al., 2012) which is the key to the latitudinal

358   gradient of insect diversity (Archibald et al., 2010). Leroy & Roiron (1996) indicated that

359   Bernasso paleoforest grew under temperatures of 14–15°C and precipitations around 1500 mm/a.

360   Recently, Girard et al. (2017, unpublished data) re-estimated Bernasso climate with different

361   approaches and some results, based on leaf morphological traits, estimated temperatures in

362   Bernasso to be cooler than estimations of Leroy & Roiron (1996), while the pollen analysis from

363   the same study tend to corroborate previous estimations done by Leroy & Roiron (1996).

364

365   *Relations between herbivory rates and temperatures of the coldest months*

366   Berga has a low temperature of the coldest months (from -6.4°C to 2°C) compared to

367   Willershausen which had the highest temperatures (from -0.5°C to 5.1°C) (Uhl et al., 2007;

368   Thiel, Klotz & Uhl, 2012). These lower temperatures during the cold period could explain the

369   lowest damage frequency observed in Berga. Indeed, insects are poikilotherms, meaning that

370   their body temperature is extremely dependent to the environment temperature (Meglitsch,

371   1972). Cooler temperatures decrease the insect metabolism (leading to diapause of insects) and

372   the quantity of generations per year (Archibald et al., 2010), consequently it could also reduce

373   the herbivory rates during the year (Bale & Hayward, 2010). Concerning Bernasso, the different

374   estimations of temperatures, included the CMMT, are lower than those of Willershausen (Uhl et

375 al., 2007; Thiel, Klotz & Uhl, 2012), thus the lowest frequency of damage could also be due to a

376 lower insect metabolism in Bernasso than in Willershausen. The lowest frequency of damage in

377 Berga than in Bernasso could also be due to insect diapause in the case of coolest temperatures

378 being lower in Berga. However, the estimated temperatures of Bernasso overlap with the ones of

379 Berga (especially for the coolest temperatures) and therefore complicate any interpretations

380 about the damage frequency between these two fossil leaf assemblages.

381 Moreover, it is important to note that no data about insect richness of these different paleoforests

382 are available. Although it could be assumed that insect richness between Willershausen and

383 Berga could be similar because outcrops are geographically and temporally similar, the insect

384 richness of Bernasso could be quite different. Consequently, in cases of differences in the insect

385 faunas, the previous relation could be disturbed as some insects, such as larvae of *Thaumetopoea*

386 *pityocampa,* feed on plants during the winter season (Battisti et al., 2005; Buffo et al., 2007),

387 when others insects have no or lower activity (Hahn & Denlinger, 2007).

388

389 *More precision provided by proportion of generalized/specialized damages*

390 The comparison of plant-insect interaction between different locations or through

391 different time periods could still be upset by local disturbances (fires, floodings, etc.) or other

392 constraints (such as different soils) that are not perceptible in fossil record and could impacted

393 damage pattern in general (Currano et al., 2011; García, Castellanos & Pausas, 2016). Moreover,

394 taphonomic biases, especially fossil preservation and different excavation histories, could also

395 interfere with our analyses. For example, the damage frequency observed in fossil record could

396 be partly distorted because the damaged leaves had less chance to be preserved in the fossil

397 record than the complete and undamaged leaves (Ferguson, 2005). For all these reasons, we

398 suggested complementing analyses by comparison of the proportions of generalized and

399 specialized damage patterns.

400 Leckey et al. (2014) indicate that the proportion of generalist and specialist herbivores may

401 change between different forests because the difference of abiotic parameters (such as climate).

402 There are the lowest proportions of specialist interactions (mainly based on galling) in

403 Willershausen and Berga, and conversely the highest proportion is in Bernasso (Figure 3), this

404 may due to climatic factors (e.g., Leckey et al., 2014). Indeed, precipitation in Bernasso was

405 higher than in Willershausen and Berga (Leroy & Roiron, 1996; Uhl et al., 2007). Also, the

406   hydric seasonality was possibly more important in Bernasso as indicated by some CLAMP

407   estimations (other CLAMP estimations minimize Bernasso hydric seasonality; Girard et al. 2017,

408   unpublished data). This is also in agreement with the supposed Mediterranean climate for

409   Bernasso (based on the plant species diversity; Leroy & Roiron, 1996) that provided heavy

410   constrain to plants here due to less water availability during the dry season (Bagnouls &

411   Gaussen, 1957; Daget, 1977, 1984). The higher seasonality conditions in Bernasso compared to

412   conditions proposed for Berga and Willershausen could also be supported the idea that regional

413   conditions of Northern Atlantic realm were more marked by higher seasonality during

414   Pleistocene than the Pliocene (Williams et al., 2009; Hennissen et al., 2015; Utescher et al.,

415   2017). Water stress should have a positive impact on galling quantity, as many studies already

416   mentioned that galling is an adaptation of stressful environment (Fernandes & Martins, 1985;

417   Fernandes & Price, 1988, 1992; Price et al., 1998; Lara, Fernandes & Gonçalves-Alvim, 2002).

418   In addition, Cuevas-Reyes et al. (2003), who studied the development of galling, showed that it

419   exists a negative correlation between gall-forming insect species richness and plant species

420   richness. It could also partly explain the highest proportion of specialized interactions in

421   Bernasso. Additionally, a forest in its late successional stage, as it has been proposed for

422   Bernasso (Leroy & Roiron, 1996; Adroit et al., 2016), tend to favor the richness of gall-inducing

423   insects (that increase the proportion of specialized interaction) (Fernandes, Almada & Carneiro,

424   2010; Adroit et al., 2016).

425

426   *Inputs of the comparisons between the common plant species from the different outcrops*

427   This global comparison of specialized and generalized damages between the fossil leaf

428   assemblage of Bernasso, Willershausen and Berga are also observable precisely on the common

429   plant species statistically represented in each outcrop. However, the FFGs and especially the

430   undamaged feature on some plant taxa are similar or could be slightly different between the

431   fossil leaf assemblage (Figure 5). It tends to confirm that the abiotic parameters are important

432   determinant factors involving significant variation of herbivory between different

433   paleoenvironments (Cuevas-Reyes et al., 2004, 2003; Leckey et al., 2014). Biotic parameters can

434   also be involved in the difference of interaction structures. For example, a decrease in food

435   quality caused by higher concentration of carbon in plants could also have a negative impact on

436   herbivory (Stiling & Cornelissen, 2007), but in general, it is compensated by an increase of

437 insect feeding (Bezemer & Jones, 1998). The impact of biotic factors seems to be further

438 confirmed as in Willershausen we can note that most Fagales (Betulaceae: *Alnus, Betulus,*

439 *Carpinus*; Fagaceae: *Fagus, Quercus*; Juglandaceae: *Carya, Juglans*) are all associated to hole

440 feeding and to undamaged feature (Figure 5A). This measurement cannot be due to hazard but it

441 probably reflects an effect of some biotic parameters (such as genetic background, plant

442 competition, host specificity, etc.).

443

444 **CONCLUSION**

445 Despite their similar plant species and their relative geographical and stratigraphical proximity

446 (at least for Berga and Willershausen), trophic structures of those paleoforests were different. On

447 the contrary to different hypotheses made on previous studies (e.g., Fernandes & Martins, 1985;

448 Fernandes & Price, 1988; Coley & Aide, 1991; Coley & Barone, 1996; Price et al., 1998; Lara,

449 Fernandes & Gonçalves-Alvim, 2002; Zvereva & Kozlov, 2006; Currano, Labandeira & Wilf,

450 2010), there was no relationships between the mean annual temperature and the quantity of

451 plant-insect interactions, as well as between the mean annual precipitation and the proportion of

452 some specialist damages. The comparison of the fossil records of Willershausen, Berga and

453 Bernasso allowed discussion about the potential impacts of seasonality of the precipitation on the

454 high proportion of galling. In addition, results suggested also that the herbivory rates could be

455 impacted by the mean temperature of the coldest months of these paleoenvironments. Such

456 observations can be related to the insects' response to climatic variation, which is very sensitive

457 (Bale & Hayward, 2010). The next step should be to conduct a meta-analysis in order to improve

458 the knowledge of the relations between plant-insect interactions and climate, to this end, further

459 studies are needed. Concerning European paleoforests, the studies of other late Pliocene outcrops

460 such as the one of Frankfurt-am-Main in Germany (Thiel et al., 2012) or Fossano in Italy

461 (Macaluso et al., 2017) should provide interesting data in that way. Lastly, this study points out

462 that comparisons of plant-insect interactions from different paleoforests are limiting by the fossil

463 preservation which significantly affects the fossil leaf collections available in the outcrops.

464 Comparisons with some similar modern forests could be relevant in order to better discern the

465 differences in proportions of plant species in the paleoforests communities.

466

467

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767

The location of Willershausen and Berga outcrops (Germany) from the late Pliocene.

A- The location of Germany in Europe. B- The locations of both outcrops in Germany. C- Zoom on the area near Göttingen. On the scale, each dash (black or white) represents 5km. The data from the maps A and B come from Natural Earth database (http://www.naturalearthdata.com)

Origin data : Natural Earth
Coordinate system : WGS84 et ED1950utm

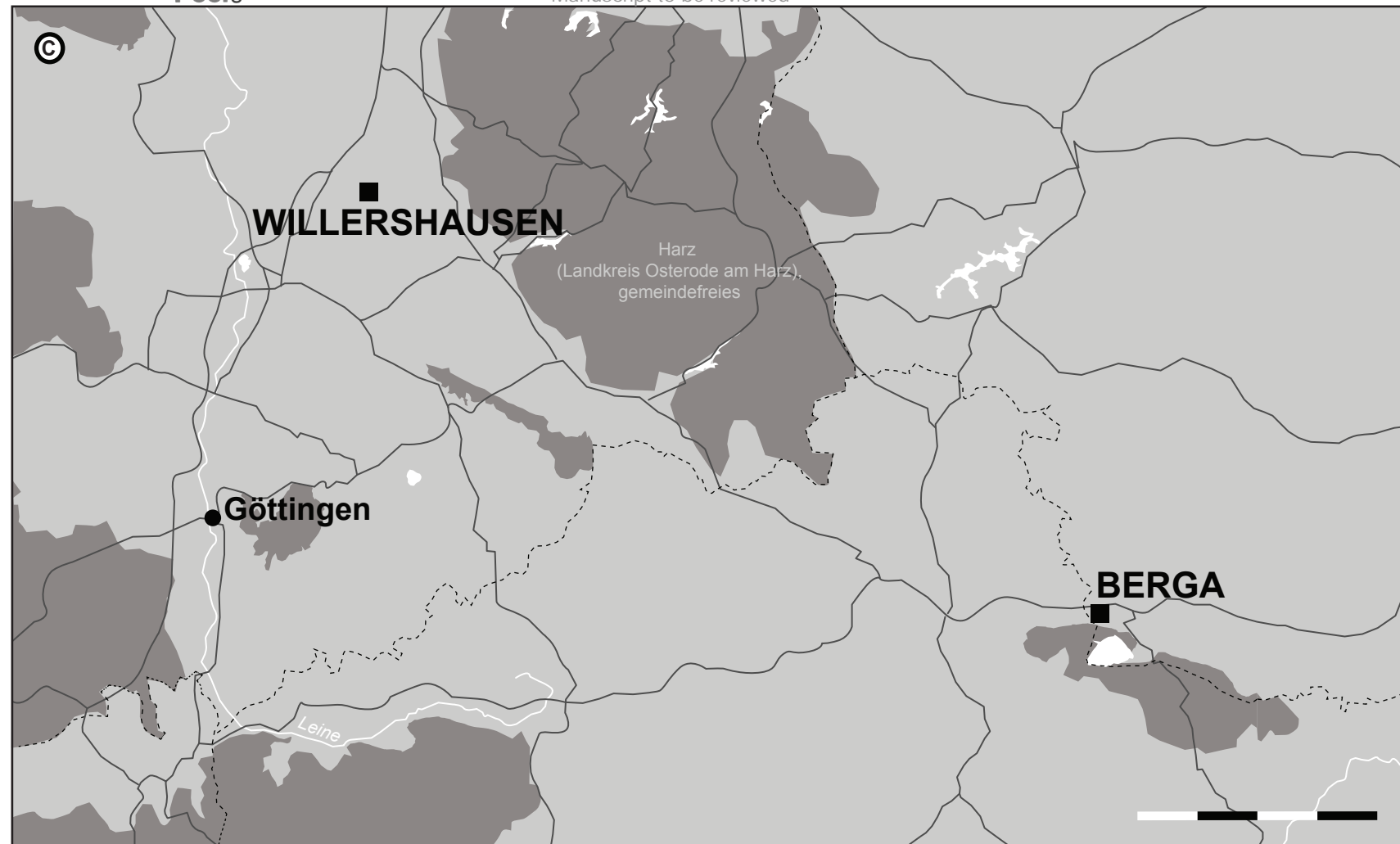
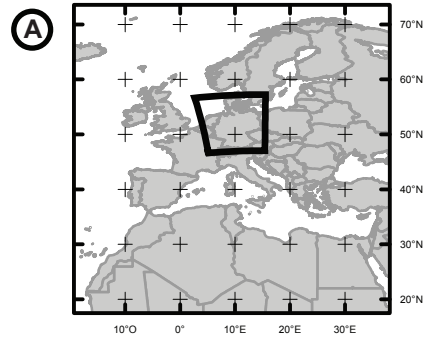



Figure 2

Well-preserved samples of fossil leaves morphotypes from Willershausen and Berga outcrops, late Pliocene from Germany.

Plate 1. Fossil leaves from Willershausen (Göttingen coll.). A- *Ulmus caprinifolia* with Hole feeding (DT05). B- *Alnus spaethii* with Margin feeding (DT14). C- *Fagus* sp with Piercing & Sucking (DT168). enlarged in 'd'. F- *Ulmus campestris* with Mining (DT109) enlarged in 'e'. G- *Quercus praeerucifolia* with Galling (DT145) enlarged in 'h'. J- *Ulmus caprinifolia* with Skeletonization (DT17). K- *Populus tremula* with Surface feeding (DT30) enlarged in 'i'.

Plate 2. Fossil leaves from Berga (Dresden coll.). L- *Cercidiphyllum crenatum*. M- *Fagus attenuata* with Hole feeding (DT01). N- *Juglans* sp. with Galls (DT34) . O- *Pterocarya paradisiaca*. P- *Quercus pseudocastanea* with Galling (DT116). Q- *Quercus castaneifolia.* White scale bar represents 1cm, black scale bar represents 0.5cm.

Photographs by Benjamin Adroit.

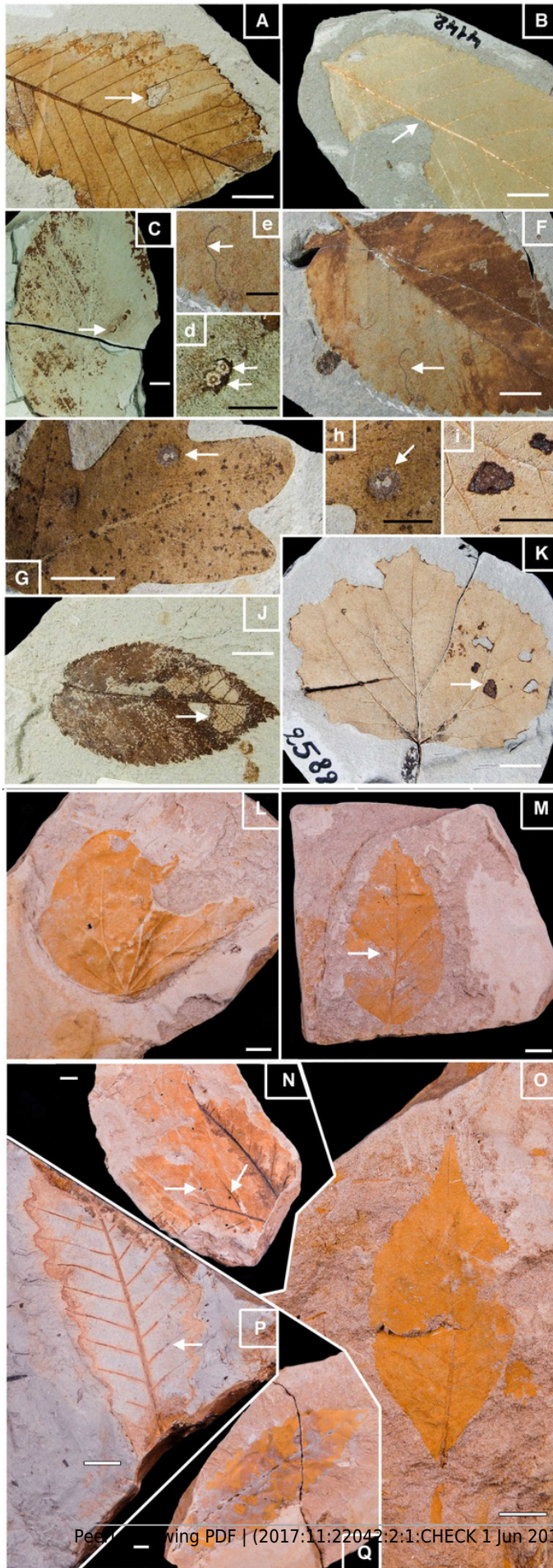



Figure 3

Quantitative distribution of plant-insect interactions from Willershausen, Berga outcrops (late Pliocene) and the fossil deposit of Bernasso (early Pleistocene).

The data of Bernasso come from the publication of Adroit et al., (2016). For each damage frequency, significant difference (alpha <0.05) from an outcrop to another one is marked by an asterisk. The percentage of generalized and specialized damages are computed only with the damaged leaves, consequently their sum on each outcrop is 100% in this figure. According to the whole amount of leaves the percentage of generalist interactions are 42.8% for Willershausen, 17.8% for Berga and 19.8% for Bernasso and the percentage of specialist interactions are 11.2% for Willershausen, 8.4% for Berga and 17.9% for Bernasso.

Number of leaves | Percent damaged | Percent generalized | Percent specialized

Hole | Margin | Skeletonization | Surface | P&S | Galling | Mining

Bernasso (-1.96 Ma)

Adroit et al., 2016

Berga (-3, -2.6 Ma)

Willershausen (-3, -2.6 Ma)

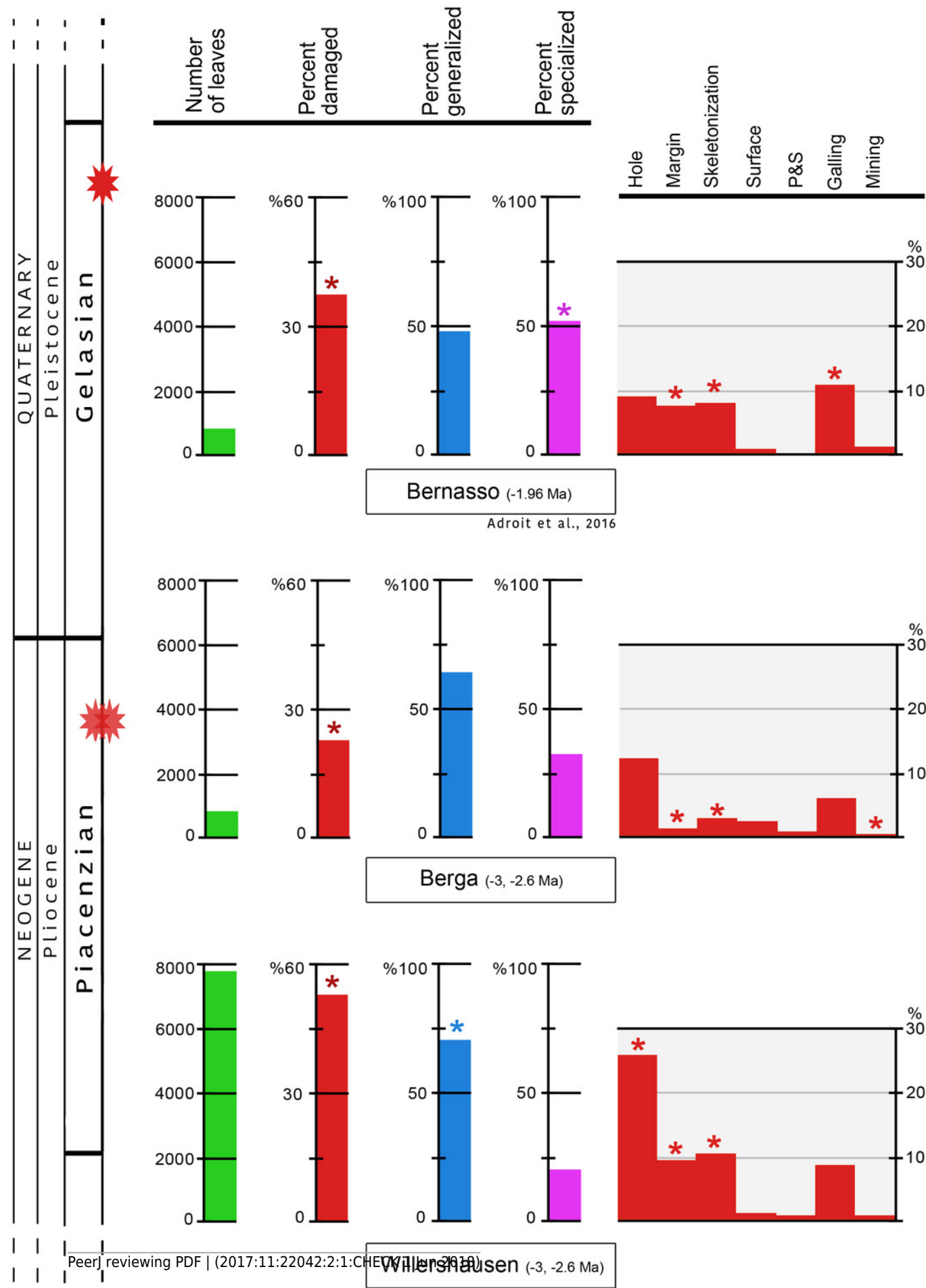
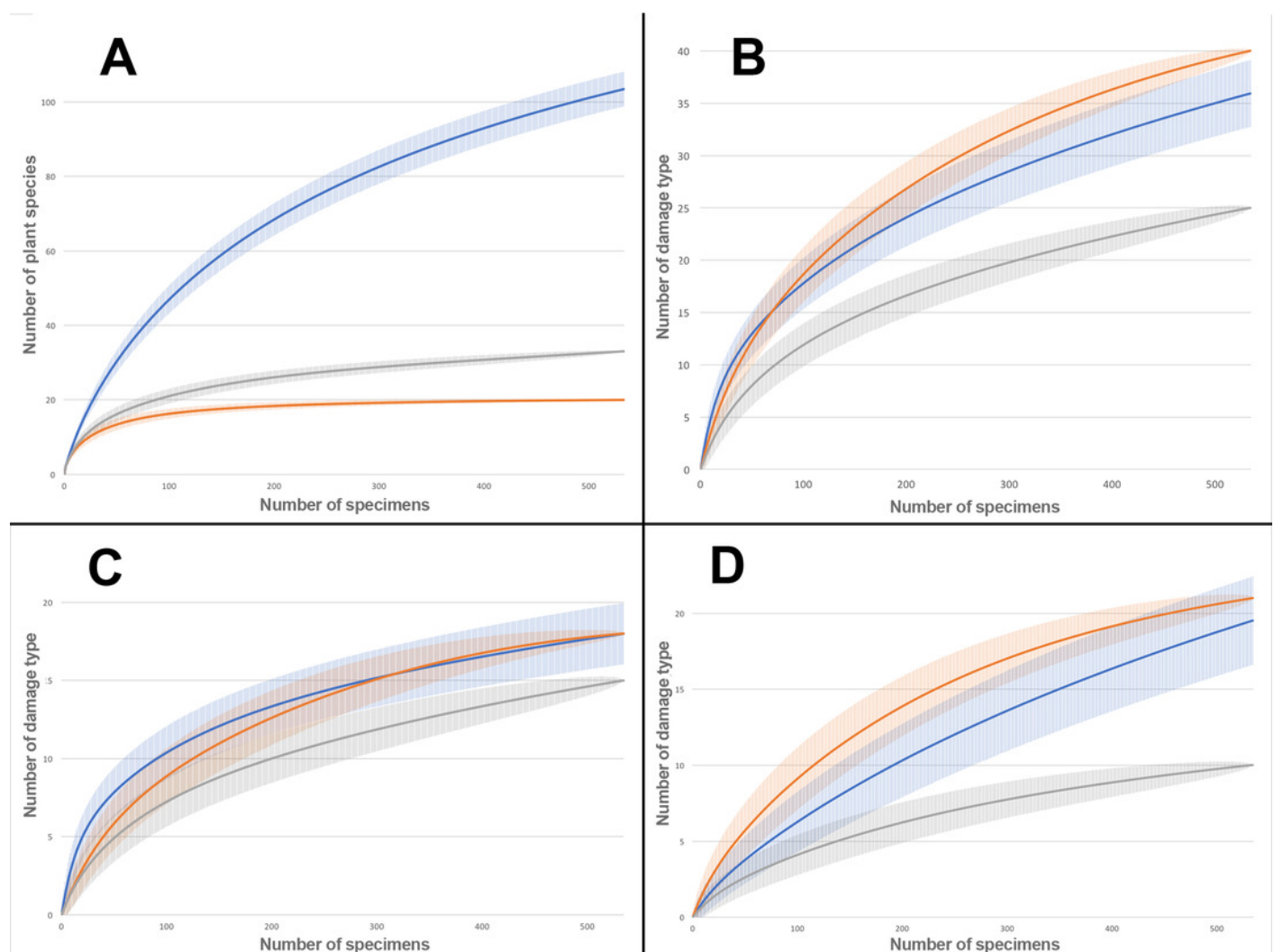
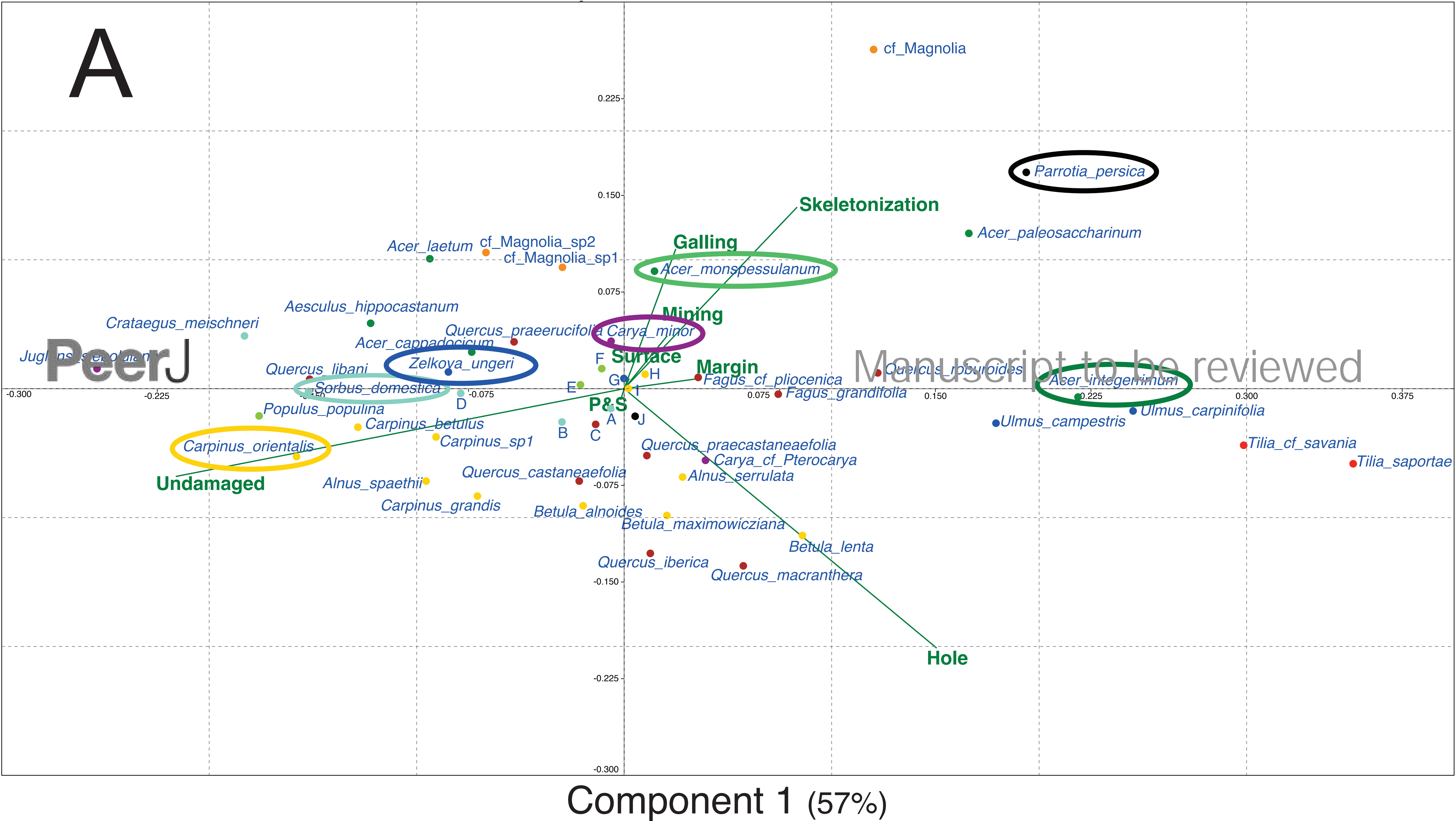
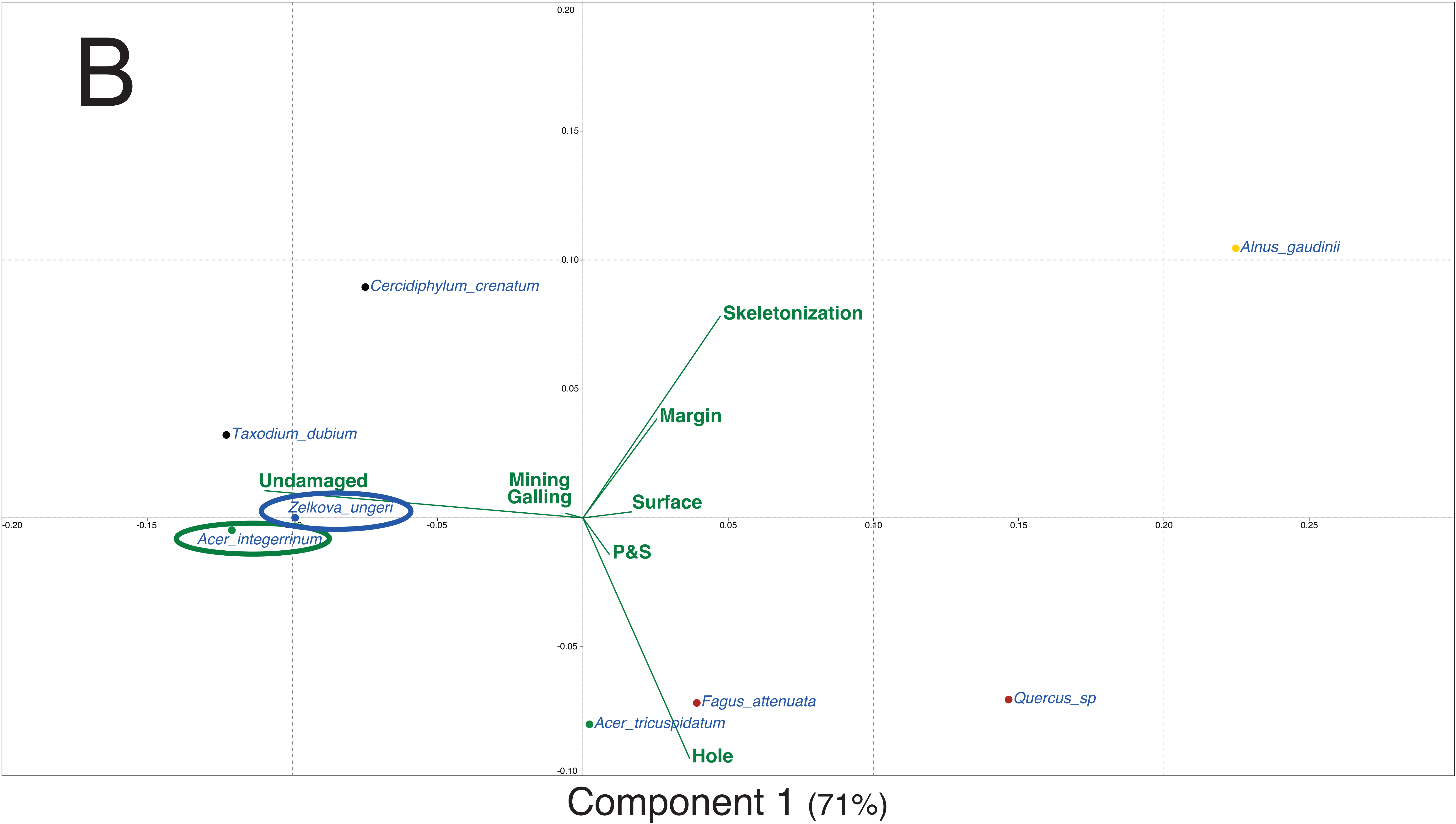
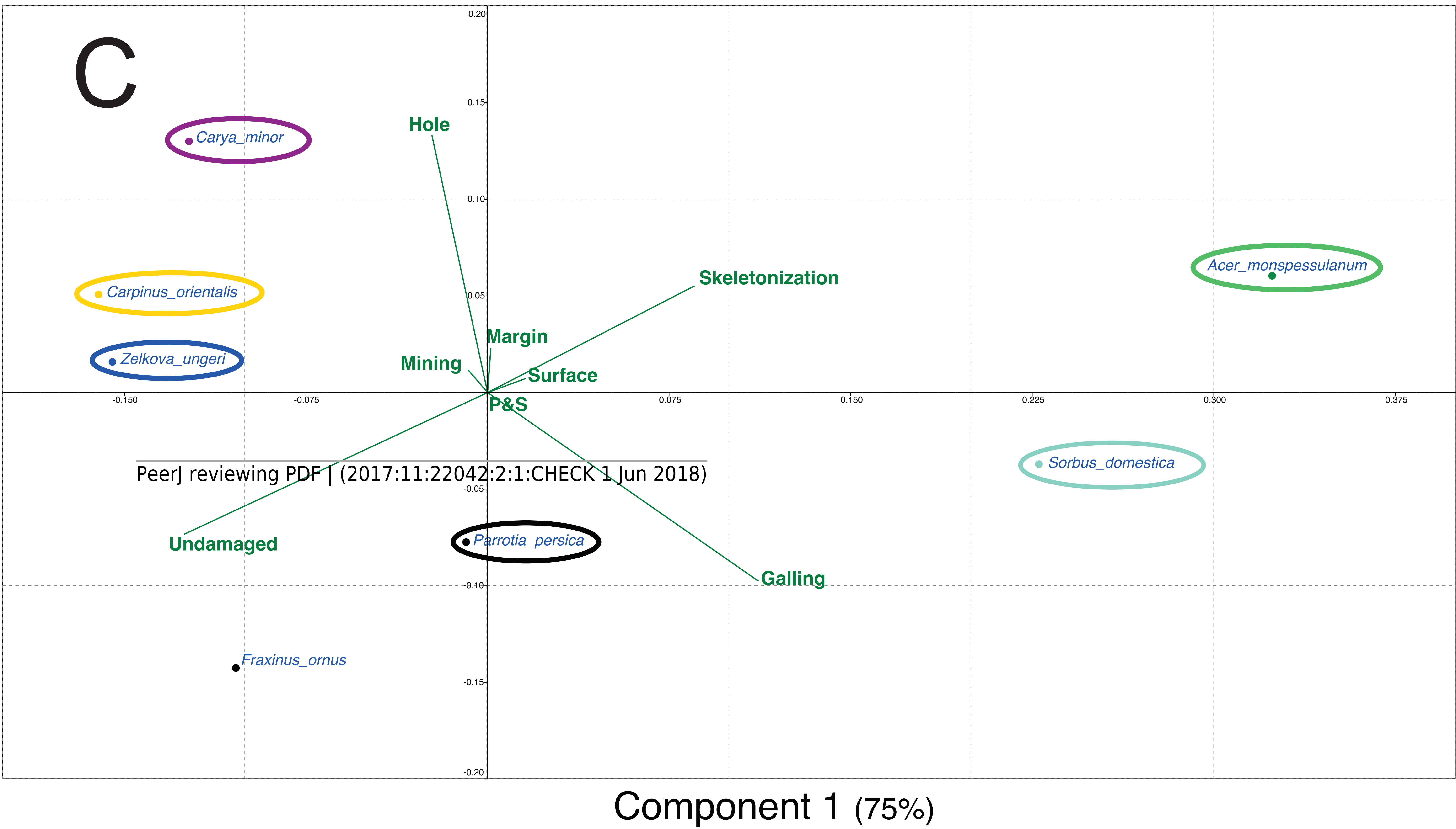



Figure 4

Rarefaction curves on the leaves from Willershausen, Berga (Germany; late Pliocene) and Bernasso (France, early Pleistocene).

Data of Bernasso come from the publication of (Adroit et al. 2016). The grey curves represent Berga, the blue curves represent Willershausen and the orange curves represent Bernasso. The shaded area represents the standard deviation below and above the average of the resamples, with the method from Heck et al. (1975). Rarefaction curves represent the number of specimens by: A- Richness of plant species; B- Richness of damage type (DT); C- Richness of generalized damage; D- Richness of specialized damage

Principal components analysis (PCA) based on the proportion (in percentage) of the FFG on each plant species.

A- Willershausen (Germany, late Pliocene), B- Berga (Germany, late Pliocene), C- Bernasso (France, early Pleistocene). Circles are used to highlight the common plant species between the different outcrops. Due to large amount of plant species names on A- Willershausen, the species names near the axes intersection were replace by alphabetic letter for visibility concerns: a- *Malus pulcherrima*, b- *Prunus mahaleb*, c- *Fagus sylvatica*, d- *Sorbus gabbrensis*, e- *Populus tremula*, f- *Populus willershausensis*, g- *Zelkova caprinifolia*, h- *Betula pubescens*, i- *Betula_sp1*, j- cf_*Toona*.

Frequency of the leaves damaged per FFG based on the whole flora.

It happens that there is more than one FFG on a leaf damaged, consequently the sum of the percent of galling, mining, margin feeding, hole feeding, skeletonization, surface feeding and piercing & sucking exceed the value of the damaged leaves. The Data for Bernasso originate from Adroit et al. (2016). Detailed of damaged leaves per species are presented in Table S3.

Outcrops	#leaves	Damaged	Generalist	Specialist	External_Specialized	Galling	Mining	MarginF	HoleF	Skeletonization	SurfaceF	P&S
WILLERSHAUSEN	7932	50.43	42.80	10.16	1.11	7.01	1.59	9.86	26.94	11.01	1.64	1.10
BERGA	534	25.09	17.79	7.12	1.31	6.18	0.19	1.87	12.73	2.62	2.06	0.94
BERNASSO	535	34.58	19.81	15.70	2.24	11.78	1.68	7.10	9.72	7.66	0.93	0.00