

# **Fossil mega- and micro-flora from Bernasso (Early Pleistocene, southern France): A multimethod comparative approach for paleoclimatic reconstruction**

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#### **Fossil mega- and micro-flora from Bernasso (early Pleistocene, southern France): a**

#### **multimethod comparative approach for palaeoclimatic reconstruction**

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#### 19 **Abstract**

20 Bernasso is an early Pleistocene locality that has a well-dated floral fossil content including both 21 pollen grains and megaremains. Pollen record shows vegetation changes corresponding to two 22 glacial and one interglacial periods. The megafloral fossil assemblage was interpreted as an 23 association of present-day Mediterranean and Caucasian taxa that developed during a late 24 interglacial stage of the beginning of the Pleistocene. In the present study, climatic reconstruction 25 using the Climate Leaf Analysis Multivariate Program (CLAMP) and the Leaf Margin Analysis 26 (LMA) methods based on the megaflora on one hand and using the Climatic Amplitude Method 27 (CAM) based on the pollen flora on the other hand have been used to check the qualitative climatic 28 estimates based on the comparison between palaeovegetation data and similar modern ecosystems. 29 CLAMP and LMA analyses show that the Bernasso climate was colder than the present-day climate 30 of Southern France and of the Hyrcanian and Euxinian regions. On contrary, CAM shows mean 31 annual temperatures higher than the modern Bernasso temperature and equivalent to the 32 temperature of the Hyrcanian and Euxinian regions. Mean annual precipitation and relative 33 humidity reconstructed by CLAMP and CAM are in agreement and indicate that the climate was 34 wetter than today at Bernasso. The differences between the three methods highlight important 35 methodological biases that could be solved by including additional calibration sites in Europe and in 36 the Mediterranean region.

37

38 **Key-words**: CLAMP analyses; Leaf Margin Analysis; Climatic Amplitude Method

#### 39 **1. Introduction**

40 Many methods for palaeoclimatic reconstruction exist such as Climate Leaf Analysis 41 Multivariate Program (CLAMP; Wolfe, 1993), Leaf Margin Analysis (LMA;Wilf, 1997), 42 Coexistence Approach (Mosbrugger and Utescher, 1997) that are generally applied on leaves 43 assemblages, the Modern Analog Technique (MAT; Guiot, 1990) used on the recent Quaternary 44 period for which modern analogues of pollen data may be found, isotopic analysis (Covey and 45 Haagenson, 1984), Climatic Amplitude Method (CAM; Fauquette et al., 1998a and b) especially 46 developed for periods without modern pollen assemblages analogues, "Climate Reconstruction 47 Analysis using Coexistence Likelihood Estimation" (CRACLE; Harbert and Nixon, 2015). Despite 48 this plethora, it appears that they cannot be used in all cases and they all present different biases that 49 affect the reliability of climatic reconstruction (*e.g.* Uhl et al., 2007a; Blonder et al., 2012; St. 50 Jacques et al., 2015). Climate reconstruction techniques based on leaf physiognomy (such as 51 CLAMP and LMA) tend to underestimate palaeotemperature (Kowalski and Dilcher 2003). Such 52 differences have been interpreted as a consequence of the fact that fossil floras are mostly 53 composed by species from wet environments (Kowalski and Dilcher 2003). Uhl et al. (2007a) 54 compared the results given by different leaf physiognomic techniques (CLAMP, LMA, CA and 55 ELPA - European Leaf Physiognomic Approach) applied on diverse Cenozoic palaeofloras. Uhl et 56 al. (2007a) highlighted that the differences between the different methods mostly depend on the age 57 of the fossil localities: differences are more marked for Neogene floras than for Palaeogene ones. 58 The same authors also showed that, in general for the Neogene, CLAMP provides lower estimation 59 of temperature than CA and ELPA. Blonder et al. (2005) also highlighted a possible bias for leaf 60 physiognomy methods. Before fossilisation, leaves fall on the grounds where death processes 61 happen. In particular, Blonder et al. (2005) studied shrinkage and showed that it can strongly affect 62 leaf area (reduction of 22% of the leaf area in average on the 3401 studied leaves, with a maximum 63 of 82% of shrinkage). Such variation can deeply affect palaeoclimatic reconstructions based on leaf 64 physiognomy only. Concerning pollen-based climate reconstruction, St. Jacques et al. (2015) 65 showed that human activities have more or less altered the modern vegetation and hence the pollen 66 rain at least since the mid-19th century. Calibrations for palaeoclimate reconstructions based on

67 modern pollen assemblages or pollen distribution are thus somehow biased.

68 Bernasso (southern France) is a fossil locality of the Early Pleistocene (Ildefonse et al., 69 1972; Ambert et al., 1990) that yielded well-documented micro- and megaflora (Suc, 1978; Leroy 70 and Roiron, 1996). Bernasso palaeoflora was a unique plant assemblage, mixing both 71 Mediterranean and Caucasian taxa (Suc, 1978; Vernet, 1981; Leroy and Roiron, 1996). Whereas the 72 Bernasso palaeoflora is well-known, its climatic significance can be improved. In particular, we do 73 not know why a mixed vegetation developed at Bernasso at the beginning of the Pleistocene. The 74 coexistence of Caucasian and Mediterranean taxa needs specific climatic conditions: combination 75 of Caucasian climatic conditions and Western Mediterranean climatic conditions. So, in order to 76 understand the development of the atypical palaeoflora of Bernasso, Leaf Margin Analysis (LMA; 77 Wilf, 1997), Climate Leaf Analysis Multivariate Program (CLAMP; Wolfe, 1993, 1995) analysis 78 based on the megaflora and Climatic Amplitude Method (CAM; Fauquette et al., 1998a and b) 79 based on pollen data were applied to mega- and micro-fossil assemblages to better characterise the 80 climatic conditions that controlled the vegetation development in the Bernasso area at the beginning 81 of the Pleistocene. This study has provided an opportunity to compare climatic estimates obtained 82 through three methods based on leaf material and pollen data and to make quantitative instead of 83 qualitative reconstructions.

84

- 85 **2. Material and methods**
- 86 *2.1. Site of Bernasso*

87 The fossil locality of Bernasso (43°43'34"N, 3°15'25"E) is located in the northwestern part 88 of the Hérault Department (southern France), at an altitude of 512 m asl (Fig. 1). Today, at Lunas 89 (altitude of 371 m asl), the nearest small town at  $\sim$ 5 km from Bernasso (Fig. 1), the climatic 90 parameters are the following: a mean annual temperature of 12.8°C, a mean temperature of the 91 coldest month of 4.8°C, a mean temperature of the warmest month of 21.3°C, a mean annual 92 precipitation around 720 mm (source: https://fr.climate-data.org). 93 The site of Bernasso represents lacustrine deposits (diatomites and clays) that formed in the

94 volcanic context of the Escandorgue Massif that was active at the end of the Pliocene and beginning

95 of the Pleistocene. As a consequence of this volcanic activity, a basalt flow completely blocked a 96 palaeovalley, which resulted in the formation of the lake of Bernasso in which pollen grains and 97 fossil leaves were preserved. For more details about the geological and sedimentological contexts, 98 see following references: Ildefonse, 1970; Ildefonse et al., 1972, 1976; Suc, 1978; Ambert et al., 99 1990; Leroy, 1990; Leroy and Seret, 1992; Roiron, 1992; Leroy and Roiron, 1996.

100

101 Ambert et al. (1990) and Løvlie et al. (1992) showed that the entire succession belongs to a reverse 102 palaeomagnetic time-interval between the end of the Matuyama period (top of normal Chron 103 C2an.1n at 2.581 Ma; Hilgen et al., 2012) and beginning of the normal Olduvai episode (base at 104 1.945 Ma; Hilgen et al., 2012), dates confirmed by K-Ar dating. Considering this chronological 105 frame, Leroy and Roiron (1996) placed the Bernasso floral succession within the oxygen isotope 106 stratigraphic interval MIS 81 to 73. Suc and Popescu (2005) refined the correlation with the oxygen 107 isotope stratigraphy with respect to the feature and amplitude of climatic changes suggested by the 108 pollen record: the Bernasso floral succession spanned MIS 82 to MIS 78, i.e. from about 2.2 to 2.1 109 Ma. On the basis of pollen record (Suc, 1978), three successive pollen zones have been defined 110 illustrating different vegetation types: the oldest (pollen zone I) and the youngest (pollen zone III) 111 are dominated by a steppe assemblage and correspond to dry and/or cold periods, the intermediate 112 zone (pollen zone II) indicates a vegetation that developed under a wetter and warmer climate (Fig. 113 2). By comparison to regional pollen diagrams and to other glacial-interglacial cycles, pollen zone 114 II has been attributed to the last part of an interglacial when the climate was already cooling down, 115 but with still relatively high precipitation (Leroy, 1990, 2007). Outcrop collapse did not allow 116 sampling the transition from pollen zone I to pollen zone II; hence a hiatus is recognised despite the 117 attempt to fill it in by making a borehole (core BNI). Pollen zones I and III provided only pollen, 118 while in one horizon of pollen zone II an abundant megafloral assemblage including leaves and 119 fruits has been found (Ildefonse et al., 1976) (Fig. 2).

120

121 *2.2. The fossil megaflora*

122 More than 800 fossil leaves, attributed to 23 different taxa (for details, see Leroy and

123 Roiron, 1996), were collected at Bernasso. These leaves are now preserved in the Collection of the 124 Institut des Sciences de l'Evolution of Montpellier (ISEM) (contact:

125 vincent.girard@umontpellier.fr) and form part of the palaeontological Collection of the University 126 of Montpellier (https://collections.umontpellier.fr/; contact: suzanne.jiquel@umontpellier.fr). When 127 the present study was conducted, the collection was split into two sub-collections, one housed in the 128 ISEM, one housed in the former Centre de Bioarchéologie et d'Ecologie of Montpellier. The 129 present study was conducted on this second sub-collection that represents only 590 leaves. No 130 particular selection was done to partition the collection and the subset used in this study contains all 131 the species and morphological variants found at Bernasso.

132 The most abundant megaremains correspond to the species *Carpinus orientalis* Mill.,

133 *Parrotia persica* (DC) C.A. Mey., and the genera *Acer* L. and *Carya* Nutt. The other leaves are

134 mostly angiosperms (15 taxa) and only few correspond to ferns (1 taxa) and gymnosperms (only 2

135 needles). For more details about this fossil assemblage, see Leroy and Roiron (1996) and the

136 Appendix.

137

#### 138 *2.3. The fossil microflora*

139 On the basis of pollen record (Suc, 1978), three successive pollen zones have been defined 140 illustrating different vegetation types: the oldest (pollen zone I) and the youngest (pollen zone III) 141 are dominated by a steppe assemblage and correspond to dry and/or cold periods, the intermediate 142 zone (pollen zone II) indicates a vegetation that developed under a wetter and warmer climate (Fig. 143 2). Unfortunately, a hiatus has been identified between pollen zones I and II. By comparison to 144 regional pollen diagrams and to other glacial-interglacial cycles, pollen zone II has been attributed 145 to the last part of an interglacial when the climate was already cooling down, but with still relatively 146 high precipitation (Leroy, 1990, 2007). Pollen zones I and III provided only pollen, while the 147 abundant megafloral assemblage including leaves and fruits has been found in pollen zone II 148 (Ildefonse et al., 1976) (Fig. 2).

149 In the present study, we focus on the three pollen records covering pollen zone II: 1) the 150 megaflora layer itself (Leroy and Roiron, 1996), 2) the outcrop studied by Suc (1978) and 3) the

151 borehole BN I (Leroy and Roiron, 1996) (Fig. 2). The taxa recorded are given Table S2. In pollen 152 zone II, pollen data point to an environment dominated by a vegetation rich in arboreal taxa such as 153 *Carya*, *Pterocarya*, *Celtis*, *Ulmus-Zelkova*, *Carpinus orientalis*, *Acer*, *Parrotia persica*, *Alnus*, etc. 154 Gymnosperms like *Tsuga*, *Abies*, *Picea* are also present in the pollen data (Suc, 1978). Pollen zone 155 II has been related to a warm and wet climatic phase, but already belonging to the end of an 156 interglacial (Suc, 1978; Leroy, 1990; Leroy and Roiron, 1996; Leroy, 2007). 157 Based on these pollen data, palaeoclimate has been reconstructed using the Climatic 158 Amplitude Method (CAM) in which the climatic requirements of modern taxa are transposed to 159 fossil data (Fauquette et al., 1998a, b). This approach relies on the relationship between the relative 160 pollen abundance of each individual taxon and climate. It takes into account not only 161 presence/absence criterion as in the Coexistence Approach (Utescher and Mosbrugger, 1997) but 162 also pollen percentages to provide more reliable reconstructions. 163 The most probable climate for a fossil pollen assemblage is estimated as the climatic interval 164 in which the highest number of taxa can exist and a "Most Likely Value" (MLV) corresponding to a

165 weighted mean according to the size of the climatic intervals of all taxa. As the precision of the 166 information obtained from a taxon's climatic interval is inversely related to the width of this 167 interval, the weights are greater for taxa with smaller intervals (for more details, see Fauquette et 168 al., 1998a).

169 *Pinus* pollen grains are excluded from the pollen sum as they are generally over-represented 170 due to a prolific production and over-abundance due to long-distance transport. Moreover, at the 171 genus taxonomic level, the various species of *Pinus* develop under a large variety of climates and 172 *Pinus* is thus not really informative. High latitude/altitude taxa are also excluded from the 173 reconstruction process to avoid a cold bias linked to pollen transport from higher elevations 174 (Fauquette et al., 1998a). The excluded taxa are defined on the basis of their occurrence in modern 175 vegetation zones (Fauquette et al., 2018): vegetation types described for the Neogene and lower 176 Pleistocene are found today in the Southeastern China (Wang, 1961; Hou, 1983). There, the vertical 177 distribution of vegetation is characterized from the base to the top of the mountains by evergreen 178 broad-leaved forest, mixed evergreen and deciduous broad-leaved forest, *Picea*/*Tsuga* forest, *Abies* 

179 forest and high mountain meadows (Wang, 1961; Hou, 1983). In our study, this altitudinal 180 stratification and the absence of high altitude taxa in the megaflora are the base for excluding the 181 modern high altitude taxa from the climatic reconstruction in order to infer the low to middle-low 182 altitude climate (Fauquette et al., 1998a). Comparison with other diatomite palaeofloras justifies 183 also this choice. Indeed at Murat (Roiron, 1991), the high percentages of *Abies* pollen grains in the 184 diatomites are associated with *Abies* megafossils (leaves and reproductive structures), which is not 185 the case at Bernasso.

#### 186

#### 187 *2.4. Methods used for palaeoclimatic quantification*

188 The Leaf Margin Analysis (LMA) is used to provide mean annual temperature estimates. It 189 was applied on the leaves of Bernasso with the different equations derived by Wolfe (1979), Wilf 190 (1997), Gregory-Wodzicki (2000), Greenwood et al. (2004), Traiser et al. (2005) and Miller et al. 191 (2006) for the different regions of the globe. The only one available for Europe (Traiser et al., 2005) 192 is compared to other equations of the Northern Hemisphere, as it is the equation with the weakest 193 correlation between the mean annual temperature and proportion of entire margined leaves (Traiser 194 et al., 2005).

195 The Climate Leaf Analysis Multivariate Program (CLAMP) was also applied to the fossil 196 leaves of Bernasso. The online CLAMP application was used for this purpose (Wolfe, 1993, 1995; 197 http://clamp.ibcas.ac.cn/). Since its initial introduction, CLAMP has undergone continuing 198 development (Kovach and Spicer, 1995; Wolfe and Spicer, 1999; Spicer, 2007, 2008; Spicer et al., 199 2004, 2009) including an online version (Yang et al., 2015) and now encompasses 32 200 morphological characters of leaves concerning the shape and size of the leaf, its margin (presence or 201 absence of teeth), the shape of the leaf apex, base and surface. For each morphological character, a 202 value between 0 and 1 was attributed according to standard CLAMP protocols. All the data were 203 summarized in a matrix (Table S1). This was then analysed with the CLAMP online canonical 204 correspondence analysis (CCA). We tested the different CLAMP calibrations that concern the 205 Northern and Southern hemispheres to provide ranges of values for different environmental 206 parameters from Bernasso. Two plant species from Bernasso were not taken into account (*Aspidium* 

207 *meyeri*, Filicophyta and *Ceratophyllum demersum*, Ceratophyllaceae, aquatic angiosperm) because 208 they do not correspond to woody dicotyledon angiosperms. CLAMP does not provide directly mean 209 annual precipitation data but precipitation during the three wettest and driest months, which allow 210 estimating the mean annual precipitation. It can be estimated as an interval comprised between a 211 low value of precipitation (LMP) and a high value (HMP). The low value can be calculated as 212 follows:

213 LMP =  $3 \times 3DRY + 3WET(1)$ 

214 where 3DRY corresponds to the precipitation during the three driest months and 3WET to the 215 precipitation during the three wettest months. The high value can be calculated as follows: 216 HMP =  $3DRY + 3x3WET(2)$ .

217 LMP and HMP provide an interval of precipitation between a very low value (nine "dry" months 218 and three "wet" months) and a very high value of precipitation (three "dry" months and nine "wet" 219 months), the real mean annual precipitation being between these two extreme values.

220 Using pollen data, palaeoclimate is reconstructed with the Climatic Amplitude Method 221 (CAM) in which the climatic requirements of modern taxa are transposed to fossil data (Fauquette 222 et al., 1998a, b). This approach relies on the relationship between the relative pollen abundance of 223 each individual taxon and climate. It takes into account not only presence/absence criterion as in the 224 Coexistence Approach (Mosbrugger and Utescher, 1997) but also pollen percentages to provide 225 more reliable reconstructions.

226 The most probable climate for a fossil pollen assemblage is estimated as the climatic interval 227 in which the highest number of taxa can exist and a "Most Likely Value" (MLV) corresponding to a 228 weighted mean according to the size of the climatic intervals of all taxa. As the precision of the 229 information obtained from a taxon's climatic interval is inversely related to the width of this 230 interval, the weights are greater for taxa with smaller intervals (for more details, see Fauquette et 231 al., 1998a).

232 *Pinus* pollen grains are excluded from the pollen sum as they are generally over-represented 233 due to a prolific production and over-abundance due to long-distance transport. Moreover, at the 234 genus taxonomic level, the various species of *Pinus* develop under a large variety of climates and

235 *Pinus* is thus not really informative. High latitude/altitude taxa are also excluded from the 236 reconstruction process to avoid a cold bias linked to pollen transport from higher elevations 237 (Fauquette et al., 1998a). The excluded taxa are defined on the basis of their occurrence in modern 238 vegetation zones (Fauquette et al., 2018): vegetation types described for the Neogene and Early 239 Pleistocene are found today in the Southeastern China (Wang, 1961; Hou, 1983). There, the vertical 240 distribution of vegetation is characterized from the base to the top of the mountains by evergreen 241 broad-leaved forest, mixed evergreen and deciduous broad-leaved forest, *Picea*/*Tsuga* forest, *Abies*  242 forest and high mountain meadows (Wang, 1961; Hou, 1983). In our study, this altitudinal 243 stratification and the absence of high altitude taxa in the megaflora are the base for excluding the 244 modern high altitude taxa from the climatic reconstruction in order to infer the low to middle-low 245 altitude climate (Fauquette et al., 1998a). Comparison with other diatomite palaeofloras justifies 246 also this choice. Indeed at Murat (Roiron, 1991), the high percentages of *Abies* pollen grains in the 247 diatomites are associated with *Abies* megafossils (leaves and reproductive structures), which is not 248 the case at Bernasso.

249

#### 250 **3. Results**

251 The results obtained with the three methods (LMA, CLAMP, CAM) are summarized in 252 Figures 3 (temperatures) and 4 (precipitation) and are detailed in Tables S3 (LMA results), S4 253 (CLAMP results) and S5 (CAM results) of the Supplementary material. Figure S1 shows CLAMP 254 CCA plots of physiognomic space obtained with the global CLAMP calibration.

255

256 In what concerns CLAMP, we only retain in the discussion the results obtained with the 257 calibrations from the Northern Hemisphere. Indeed, Calib 5 to Calib 7 correspond to calibrations for 258 Asian vegetation that suffer strong monsoonal climate (Calib5 and Calib6) and for 378 sites from 259 all continents (Calib7). Due to their specificity, these last three calibrations were not analyzed in our 260 study, but the complete CLAMP calibrations are however given Supplementary Table S4. 261 Regarding CAM, the climatic conditions reconstructed from pollen data of the leaf level and

262 of pollen zone II data (borehole BN I and outcrop) are summarized in the Supplementary Table S5.

263 Concerning Bernasso mean annual temperatures, CLAMP and LMA show similar ranges 264 but are lower than that reconstructed by CAM. In the same way, CLAMP indicates once again 265 largely lower values for mean temperature of the coldest and warmest months than CAM (Fig. 3). 266 However, mean annual precipitation and relative humidity are in agreement between both methods 267 (Fig. 4 and Supplementary Table S4 and S5), even if precipitation is slightly higher with CLAMP 268 than with CAM.

269

#### 270 **4. Discussion**

271 Comparison to modern floras highlighted similarities between the Bernasso palaeoflora and 272 the modern flora of the Hyrcanian (South of the Caspian Sea) and the Euxinian (East of the Black 273 Sea) regions (Ambert et al., 1995; Leroy and Roiron, 1996). The climate under which Bernasso 274 palaeovegetation grew was thus interpreted by these authors, by comparison with modern climatic 275 data from diverse stations of the Caucasian region (Table S6; see also Table 4 in Leroy and Roiron, 276 1996 ), as similar to that of both regions, i.e. characterised by a mean annual temperature of 14- 277 17°C, temperature of the coldest month of  $5-6.5$ °C and of the warmest month of 24-26.5°C and 278 high precipitation (more than 800 mm per year). The Bernasso palaeoflora was estimated to have 279 thus developed under a warmer  $(+ 2 \text{ to } 5^{\circ}\text{C})$  and wetter (at least  $+ 300$  to 500 mm per year) climate 280 than today. However, the presence of taxa such as *Carpinus orientalis*, *Acer monspessulanum* L., 281 *Celtis australis* L., *Fraxinus ornus* L. in the megaflora (Leroy and Roiron, 1996) and *Olea*, 282 *Phillyrea*, *Phlomis* cf. *fruticosa* and *Quercus ilex*-type in the pollen flora (Suc, 1978), suggests that 283 the Bernasso palaeoflora was exposed to climatic conditions that made the occurrence of typical 284 Mediterranean sclerophyllous elements possible in the underneath vegetation belt (Suc, 1978). 285 Leroy and Roiron (1996) could have thus misjudged the Bernasso palaeoclimate. 286

287 *4.1. Comparison of the results obtained with the three methods*

288 CLAMP and LMA indicate mean annual temperatures largely lower than the modern 289 temperatures observed in the Caucasian region and Lunas near Bernasso (Fig. 3). The CAM values 290 are furthermore in agreement with the temperatures deduced by Leroy and Roiron (1996) and are

291 close to the modern Hyrcanian values. Compared to the Hyrcanian region (that Leroy and Roiron 292 (1996) supposed to be a modern analogue to the fossil flora of Bernasso), all the methods provide 293 very low temperature values of the coldest month (Fig. 3 and Table S6). Concerning this parameter, 294 the very low temperature values with CLAMP  $(-3.79$  to  $-6.33^{\circ}$  C) seem to be incompatible with the 295 presence of mesothermic Mediterranean sclerophyllous taxa (Leroy and Roiron, 1996) that do not 296 support such negative temperatures (Quézel and Médail, 2003) (Fig. 3 and Table S6). Concerning 297 the temperature of the warmest month, CLAMP values (17.18 to 19.19 $\degree$ C) are also very low 298 compared to those of the supposed analogue of the Hyrcanian region. On the contrary, CAM values 299 for this parameter of more coherent with those of the supposed analogue of the Hyrcanian region 300 (Fig. 3 and Table S6).

301 Precipitation results are included in the precipitation range of the Hyrcanian region that has 302 precipitation varying at low altitude from 500-600 mm to the East to 2000-2200 mm to the West 303 (Fig. 4 and Table S6; Molavi-Arabshahi et al., 2016; Iran Meteorological Organization (IRIMO), 304 http://www.irimo.ir/eng/). Leroy and Roiron (1996) suggested that the Bernasso palaeoflora grew 305 under a humid (perhaps very humid) climate and our results tend to support their hypothesis. 306 However, Suc (1978) and Leroy and Roiron (1996), on the basis of pollen data, interpreted the 307 climate of the pollen phase II (during which megaremains were deposited) as warm and wet with 308 only a weak hydric seasonality (weak decrease in summer precipitation). CLAMP results do not 309 completely confirm this last conclusion and show that the hydric seasonality could have been well-310 marked, with a ratio between wettest and driest months according to some CLAMP calibrations 311 varying from ca. 1.4:1 to 5.7:1. The question of a possible seasonal drought is still unsolved, 312 especially as the CLAMP global calibration places Bernasso between East Asian (excluding 313 Southeastern), North American and Russian localities (Supplementary Figure S1). It could have 314 been the case as it is today in the Mediterranean region, but no reconstruction, whatever the method 315 used, allows to confirm it and no leaf specimen from Bernasso displays obvious functional 316 adaptation to drought.

317

318 In the CAM, high latitude-altitude taxa are excluded from the reconstruction process to

319 avoid a cold bias linked to pollen transport from higher vegetation belts. The Bernasso valley was a 320 long, narrow and steep-sided valley (Ildefonse et al., 1972), as today, and the palaeoforest was 321 growing from the lake level to the plateau at the top of the canyon. Suc (1978) and Leroy and 322 Roiron (1996) suggested that an altitudinal zonation of the palaeoflora existed. They advocated that 323 the mid-altitudinal mixed forest on the slopes of the Bernasso palaeocanyon was composed of 324 *Carpinus orientalis*, *Parrotia persica* and several species of *Acer*. *Carya* and *Zelkova* should have 325 grown only in sheltered areas of the palaeocanyon. Gymnosperms like *Tsuga*, *Abies*, *Picea* are 326 present in the pollen flora (Suc, 1978), but they were certainly not growing close to the lake 327 shoreline as no leaf or cone specimens have been found in the megaflora (Leroy and Roiron, 1996). 328 These pollen grains certainly came from a conifer forest growing at higher altitude. Such an 329 altitudinal zonation may be found today in the Southern Caspian Sea region (Rmezani et al., 2013) 330 even if it is incomplete as *Abies*, *Picea* and *Tsuga* are absent there. It may be also found in China, in 331 the subtropical evergreen broad-leaved forest region (Hou, 1983).

332 However, taking into account the high percentages of *Tsuga* in the pollen data (Suc, 1978; 333 Leroy and Roiron, 1996), although this taxon is considered as a middle to high altitude-latitude 334 taxon and is not present in the megaflora (Leroy and Roiron, 1996), we could hypothesize that 335 *Tsuga* was developing relatively close to the lake, as it was in the case of a nearby maar lake in 336 Nogaret (1.9 Ma; Brugal et al., 1990; Leroy and Seret, 1992). We thus made a climate 337 reconstruction with the CAM including the climatic requirements of this taxon. The obtained 338 temperatures are lower than the temperatures estimated excluding *Tsuga*: mean annual temperatures 339 are between 11 and 15 $^{\circ}$ C (most likely value ~13 $^{\circ}$ C), mean temperature of the coldest month 340 between -2 and  $5^{\circ}$ C (most likely value  $\sim$ 1.3°C), mean temperature of the warmest month between 341 23 and 27°C (most likely value ~24.8°C). Remarkably, mean annual precipitation and relative 342 humidity are equivalent to values without *Tsuga*. In any case the temperatures are never as low as 343 the CLAMP and LMA temperatures.

344 Adroit et al. (2016) supported the fact that the Bernasso flora should have existed under a 345 high mean annual precipitation on the basis of the low diversity and high quantity of galling found 346 on the fossil leaves of Bernasso. However, the analysis of plant insect interactions on modern and

347 fossil leaves of *Parrotia persica* made a likely correlation between mean annual precipitation and 348 proportions of insect damages on leaves unclear (Adroit et al., 2018). More in-depth analyses are in 349 development to improve our understanding of the links between plant-insect interactions and 350 climatic parameters. These future results will possibly help clarifying Bernasso palaeoclimate.

351

#### 352 *4.2. Potential and limits of the methods*

353 The three methods used in the present study have the advantage to give quantitative instead 354 of qualitative reconstruction of the climate that allows direct comparisons. However, each 355 quantitative methodology proposing climatic reconstructions based on biological palaeoproxies has 356 its own characteristics influencing the results. For the CAM, we decided to exclude the high 357 altitude/latitude taxa based on their absence in the megaflora and on their modern altitudinal 358 distribution. However, reconstructions based on leaf physiognomy (LMA, CLAMP) methods give 359 generally lower temperature values when applied to European palaeobotanical records (e.g. 360 Utescher et al., 2000; Uhl et al., 2003, 2006, 2007a, b; Peppe et al., 2011; Thiel et al., 2012). In fact 361 the modern datasets used to calibrate the relationships does not originate from modern floras 362 distributed evenly worldwide (Yang et al., 2015). For CLAMP, the available modern data sets do 363 not include data from southern Europe (only five sites in the United Kingdom and some sites in 364 western FSU; Yang et al., 2015), nor from the Middle East (http://clamp.ibcas.ac.cn). This may be 365 problematic as many authors have shown that temperate floras of different continents may have 366 different leaf-climate relationships (e.g. Gregory-Wodzicki, 2000; Greenwood et al., 2004) that led 367 to significant differences in temperature estimates up to 5°C or more (Jordan, 2011). The case of the 368 Bernasso megaflora falls into this gap as it is composed of a mixture of taxa living today in the 369 Mediterranean region and in the SW Asia (including the Hyrcanian region). However Yang et al. 370 (2015) showed that, in general, CLAMP results seem to be more influenced by climate than 371 biogeography, corroborating CLAMP results. Also CLAMP calibrations have been validated 372 against isotopic methods and found to give reliable results, in any case for the Early Eocene and 373 Miocene periods in the Southern Tibet (Xu et al., 2017 and references therein).

374 Moreover, the modern dataset incorporates many types of sites including dry land sites 375 although fossil megafloras are predominantly deposited in wet land sites, which also leads to 376 discrepancies. Due to this bias, Kowalski and Dilcher (2003) indicated that physiognomic methods 377 lead to an underestimation of temperatures between 2.5 and 10°C. With the results obtained by 378 CLAMP and LMA methods in this study, one can note that the low temperatures estimated for 379 Bernasso are found today in southern Norway, at Kristiansand (58°09'N, at sea level, source: 380 https://fr.climate-data.org) (Fig. 3; Table S6). Applying the range of underestimation of the mean 381 annual temperature given by Kowalski and Dilcher (2003), we obtain a corrected mean annual 382 temperature for Bernasso comprised between 7°C and 17°C, a range of mean annual temperature 383 more consistent with the results given by the CAM, even if it is still slightly lower. 384 Peppe et al. (2011) made reconstructions using "digital leaf physiognomy" calibrated on 385 modern data corresponding to 92 sites sampled in biomes where fossil leaves are generally 386 preserved. The correlations between the chosen leaf characters and climate parameters show that 387 these physiognomic variables are linked to climate. When applying this calibration to fossil floras, 388 the authors obtained a warmer and wetter climate, similar to that estimated by other independent 389 palaeo-proxies (Peppe et al., 2011). However, it is noteworthy that improvements can be made, 390 particularly in adding more modern sites to have a larger training set of data in calibrating the 391 model with additional sites that are still absent from this database (for instance from Europe and 392 Africa, Peppe et al., 2011).

393 All these evidences indicate that the CLAMP method seems not optimal to reconstruct the 394 climate of the early Pleistocene in Europe because of the lack of modern data to calibrate the 395 method on European ecosystems (Uhl et al., 2007a). These results are thus opposite to those of Xia 396 et al. (2009) and Bondarenko et al. (2013) in which CLAMP analyses provided coherent results 397 with other palaeoclimate reconstruction methods. CLAMP results from Bernasso probably reflect 398 the absence of modern analogues from Mediterranean climate in CLAMP. At Bernasso, 399 palaeotemperatures estimated by CLAMP and LMA are lower than those given by CAM. Also, as 400 the quantification by CLAMP and LMA made on Bernasso remains unique, it is difficult to see if 401 the Bernasso results are similar to other regional results or different from them. The values obtained 402 with CAM are in agreement with the climate reconstructed at Garraf in Northern Spain for the same 403 period (Fauquette et al., 1998a). It thus appears that, at a regional scale, CAM provides coherent 404 results and may be used to reconstruct the climate at the beginning of the Pleistocene, at least in the 405 South European region.

406

#### 407 **5. Conclusions**

408 The fossil site of Bernasso is remarkable, recording both leaves and pollen grains that allows 409 applying different climatic reconstruction methods and thus comparing results. Climatic 410 reconstruction using CLAMP and LMA methods based on megaflora on one hand and using the 411 Climatic Amplitude Method (CAM) based on pollen flora on the other hand have been made to 412 check the values given in an earlier study based on the comparison between palaeovegetation data 413 and similar modern ecosystems. In the latter study, the climate at Bernasso was compared to the 414 modern climate of the Hyrcanian and Euxinian regions.

415 The comparisons of the three methods used in this study highlight their complementarity as 416 LMA and CLAMP are based on leaf physiognomy and do not take into account the taxonomy of the 417 plants, whereas CAM is based on the modern climatic requirements of the taxa present in the pollen 418 flora from the same sediment level. CLAMP provides supplementary data about precipitation 419 pattern (*e.g.* precipitation during the three wettest and driest months) that can be partly compared to 420 the CAM results (*i.e.* mean annual precipitation).

421 However, despite this possible complementarity, the results of this study highlight important 422 differences between the three methods. CLAMP and LMA analyses show that late-interglacial 423 Bernasso climate was colder than the present-day climate in Southern France and in the Hyrcanian 424 region. Against all expectations, the obtained values are found today in southern Norway (58°09'N 425 at sea level; Fig. 3; Table S6). CAM shows mean annual temperatures higher than the modern 426 Bernasso mean annual temperature and equivalent to the temperature of the Hyrcanian region. For 427 what concerns mean temperature of the coldest and warmest months, CLAMP indicates again lower 428 values than the Climatic Amplitude Method. The only parameters more or less in agreement 429 between CLAMP and CAM are mean annual precipitation and relative humidity: the climate was

430 wetter than today at Bernasso with the existence of a weak precipitation seasonality highlighted in

431 the CLAMP results.

432 The two physiognomic methods lead to an underestimation of temperatures from 2.5 to 433 10°C may be due to problems of calibration. Absence of calibration sites in Europe and in the 434 Mediterranean region are a critical problem in the underestimation by CLAMP. Calibrations are 435 also still needed for the Climatic Amplitude Method in order to have a better precision on the 436 presence of some taxa in the local flora deduced from pollen data and to get more accurate climatic 437 reconstruction.

438

439

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615 **Figure 1** – Location map of Bernasso situated in the Northwestern part of the Hérault Department 616 (Southern France).

617 **Figure 2 –** Lithology, synthesized pollen diagrams of the three pollen records and position of the 618 megaflora layer. Pollen diagrams of the exposed section refer to Suc (1978), those of the Borehole 619 BNI and Leaf outcrop refer to Leroy (1990) and Leroy and Roiron (1996).

620 **Figure 3** – Comparison of the temperatures (in °C) estimated with the three methods, in blue the 621 mean temperature of the coldest month, in black the mean annual temperature and in red the mean 622 temperature of the warmest month. For LMA and CLAMP, the darker bars correspond to the 623 estimated values and the intervals to the standard errors for LMA regression equations Reg 1, Reg 624 2, Reg 7 and Reg 8 and CLAMP calibrations Calib 1 to Calib 4. See details for LMA and CLAMP 625 calibrations in Tables S3 and S4. For CAM, the intervals correspond to the estimated climate and 626 the darker bars to the Most Likely Values (weighted means), see details in Table S5. The green 627 bands correspond to the modern annual temperature at Kristiansand in Southern Norway, Lunas in 628 Southern France (source: https://fr.climate-data.org) and in the East and West Hyrcanian regions in 629 Iran (Molavi-Arabshahi et al., 2016; source: Iran Meteorological Organization (IRIMO),

630 http://www.irimo.ir/eng/), see Table S6 for more details.

631

632 **Figure 4** - Comparison of the precipitation (in mm) estimated with four different calibrations of 633 CLAMP and with CAM. For CLAMP calibrations, the calibrations Calib1 and Calib2 correspond to 634 Physg3brcAZ calibration (based on 144 modern vegetation sites from the Northern Hemisphere 635 temperate regions; Calib2 was obtained thanks to a meteorological correction applied in Calib1); 636 the calibrations Calib3 and Calib4 correspond to Physg3arcAZ calibration (based on 173 modern 637 vegetation sites from the Northern Hemisphere including samples from areas with pronounced 638 winter cold; Calib4 was obtained thanks to a meteorological corrections applied in Calib3). Calib5 639 is an Asian calibration. For CAM, the intervals correspond to the estimated climate and the darker 640 bars to the Most Likely Values. The four yellow bands correspond to the modern annual

- 641 precipitation at Kristiansand in Norway, Lunas in Southern France (source: https://fr.climate-
- 642 data.org) and in the East and West Hyrcanian regions in Iran (Molavi-Arabshahi et al.2016; source:

643 IRIMO, http://www.irimo.ir/eng/), see Table S6 for more details.

644

#### 645 **Supplementary material**

- 646 **Figure S1**  Canonical correspondence analysis (CCA) plots showing the place of Bernasso within
- 647 the 378 vegetation sites available in CLAMP. A- axes 1-2 space; B- axes 1-3 space; C- axes 2-3
- 648 space. Climate vectors are positioned for information. MAT- mean annual temperature; CMMT-
- 649 cold month mean temperature; WMMT- warm month mean temperature; LGS- length of the
- 650 growing season; GSP- growing season precipitation; MMGSP- mean monthly growing
- 651 season precipitation; 3-WET- precipitation during the three wettest months; 3-DRY-
- 652 precipitation during the three driest months; SH- specific humidity; RH- relative humidity;
- 653 ENTHAL- enthalpy (modified from Yang et al., 2015).
- 654

655 **Table S1** - Data matrix input to on-line CLAMP analysis tool.

656 **Table S2** – List of the pollen taxa recorded respectively in the outcrop, leaf level and borehole.

657 High latitude/altitude taxa that are excluded of the climatic reconstruction process are indicated in

658 bold and taxa used to reconstruct the climate are underlined. The Mediterranean sclerophyllous taxa

659 have been also excluded because they probably inhabited a lower vegetation belt than that of the

660 Bernasso interglacial forest. The others correspond to taxa that do not give precise climatic

- 661 information because of large climatic amplitudes or to taxa for which we do not know the climatic
- 662 requirements.

663 **Table S3** – Mean Annual Temperature (MAT) obtained from the different LMA regression

664 equations considering that Bernasso megaflora contains 25.00% untoothed leaf taxa, as shown in

665 Table S1. Reg 1, Reg 2, Reg 7 and Reg 8 are reported on Figure 3.

- 666 **Table S4** CLAMP results obtained from the Bernasso megaflora. Calib 1 to 4 are reported on 667 Figures 3 and 4.
- 668 **Table S5** Climatic estimates obtained with the Climatic Amplitude Method using pollen data of
- 669 the leaf level and of the pollen zone II (borehole BN I in Leroy and Roiron (1996) and outcrop in
- 670 Suc (1978)). Values are reported on figures 3 and 4.
- 671
- 672 **Table S6** Climatic parameters of the three modern analogues used in the discussion: the
- 673 Hyrcanian region (data obtained from Molavi-Arabshahi et al. (2016) and IRIMO
- 674 (http://www.irimo.ir/eng/), Kristiansand (Norway) and at Lunas (France) (data obtained from
- 675 Climate-data http://fr.climate-data.org).

677

# 678 Table S1



679





680









682 \* for more details about scoring of the characters: http://clamp.ibcas.ac.cn/Clampset2.html

683 Scores are the consensus of four independent scorers. CLAMP scores 32 character states; only those characters

684 represented in the Bernasso megaflora are presented here. Descriptions of all character states and the details of the

685 scoring method can be found on the CLAMP web site (http://clamp.ibcas.ac.cn).

686

687 Table S2



Picea	X	X	X
Pinaceae	X	X	X
<b>Pinus</b>	$\mathbf x$	$\mathbf X$	$\mathbf x$
Plantago	X		
Poaceae	$\mathbf X$	$\mathbf x$	$\mathbf x$
Polygonum		$\mathbf X$	$\mathbf X$
Populus	$\mathbf X$		
Potamogeton	$\mathbf X$		
Pterocarya	$\mathbf X$		
Quercus deciduous type	X		X
Quercus ilex-coccifera type	X		X
Ranunculaceae	$\mathbf x$		$\mathbf x$
Rhamnus	$\mathbf X$		
Rumex	$\mathbf X$		
Salix	X		
Scabiosa		$\mathbf X$	
<u>Tilia</u>	X		X
<b>Tsuga</b>	$\mathbf X$		$\mathbf{X}$
Typha	X		
Typha cf. latifolia		$\mathbf x$	
<b>Ulmus</b>		$\mathbf X$	
Ulmus-Zelkova type	X	X	X
Urticaceae	$\mathbf X$		X
Monoletes spores		$\mathbf X$	
Thelypteris cf. palustris			X
Selaginella sibirica			X
Osmunda			X
Sphagnum			X

<sup>688</sup> 

# 690 Table S3.



691

## 692 Table S4.



<sup>689</sup> 



693 \* The calibrations Calib1 and Calib2 correspond to Physg3brcAZ calibration (based on 144 modern vegetation sites

from the Northern Hemisphere temperate regions; Calib2 was obtained thanks to a meteorological correction applied in Calib1). The calibrations Calib3 and Calib4 correspond to Physg3arcAZ calibration (based on 173 modern vegetation sites from the Northern Hemisphere including samples from areas with pronounced winter cold; Calib4 was obtained thanks to a meteorological corrections applied in Calib3). Calib 5-7 respectively correspond to calibrations for Asian vegetations that suffer strong monsoonal climate (Calib5 and Calib6) and for 378 sites from all continents (Calib7). Due

699 to their specificity, these 3 last calibrations were not analysed in this study, but results are presented in this table. For

700 more details, see CLAMP online: http://clamp.ibcas.ac.cn/

701 MAT – Mean annual temperature; CMMT – Mean temperature of the coldest month; WMMT – Mean temperature of

702 the warmest month; GSP – Precipitation during the growing season; 3DRY – Precipitation during the 3 driest months;

703 3WET – Precipitation during the 3 wettest months; LPM – Lower estimation of the mean annual precipitation; HPM –

704 Higher estimation of the mean annual precipitation; RH – Relative humidity.

- 705
- 706 Table S5.

Parameter*	MAT $(^{\circ}C)$	MTC $(^{\circ}C)$	MTW $(^{\circ}C)$	MAP (mm)	$RH(\%)$
Leaf level	$11 - 15 - 20$	$-2 - 1.6 - 5$	$24 - 25 - 27$	$700 - 1100 - 1350$	$66 - 75 - 80$
Borehole & outcrop $ 11 - 15.5 - 20 $				$-2.2 - 3.2 - 8$ 24 - 25.7 - 27.7   700 - 1055 - 1320	$66 - 75 - 81$

<sup>707</sup> \* MAT – mean annual temperature; MTC – mean temperature of the coldest month; MTW – mean temperature of the 708 warmest month; MAP – mean annual precipitation; RH – relative humidity. All parameters are given as an interval and 709 a Most Likely Value which corresponds to a weighted mean.

- 710
- 711
- 712
- 713
- 
- 714
- 715
- 716

### 717 Table S6.



718 \* MTC - mean temperature of the coldest month; MAT – mean annual temperature; MTW – mean temperature of the

719 warmest month; DMP – precipitation during the driest month; MAP – mean annual precipitation; WMP – precipitation

720 during the wettest month.







