

## Article

# Taxonomy, Ecology and Distribution of *Juniperus oxycedrus* L. Group in the Mediterranean Basin Using Bioclimatic, Phytochemical and Morphometric Approaches, with Special Reference to the Iberian Peninsula

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**Abstract:** Several studies have been conducted in the past to clarify various aspects of species in the genus *Juniperus* L. One critical group is *Juniperus oxycedrus* L., especially from the taxonomical point of view. For this reason, we have studied the ecology, taxonomy and distribution of the taxa in the *J. oxycedrus* group. From an ecological and distribution standpoint, in this work we use the ombrodaphoxeric index (Ioex) to explain the presence of *Juniperus* populations in ombrotypes that are not optimum for these taxa. The controversy over the taxonomy of *J. oxycedrus* subsp. *badia* (H. Gay) Debeaux and *J. oxycedrus* subsp. *lagunae* (Pau ex C. Vicioso) Rivas Mart. is clarified, and it is accepted as a valid name, *J. oxycedrus* subsp. *badia*. The phytochemical differences in essential oils (EO) are addressed and their similarities analyzed; greater similarities are observed between *oxycedrus* and *badia*, and between *navicularis* Gand. and *macrocarpa* (Sm.) Ball. species. The phytochemical, molecular and distribution differences allow *J. oxycedrus* subsp. *macrocarpa* (Sm.) Ball and *J. navicularis* Gand. to be maintained as species. The results obtained make it possible to establish the rank to which the taxa belong and allow clear discrimination between species in groups that are difficult to interpret. Ecological, bioclimatic, phytochemical and morphometric similarities allow us to subordinate the subsp. *macrocarpa* to the species *J. navicularis*.

**Keywords:** bioclimatology; Cupressaceae; ecology; morphometry; phytochemistry; prickly juniper.

## 1. Introduction

In this work, we update the state of knowledge of the ecology, taxonomy and distribution of the taxa of the *Juniperus oxycedrus* group, based on our study and on research by various authors which has, occasionally, been a source of controversy.

*Juniperus oxycedrus* L. has its distribution in the Mediterranean region, from eastern Portugal to northern Iran, including Spain, the Balearic Islands, France, Corsica, Italy, Sicily, Sardinia, Northern Africa [1], Albania, Croatia, Montenegro, Serbia, Northern Macedonia, Bulgaria, Greece, Crete and Karpathos, Cyprus, Turkey, Israel and Jordan, the Crimea and Lebanon [2,3]. According to Amaral Franco [2], this species has three clearly differentiated subspecies for the Iberian Peninsula: *J. oxycedrus* L. subsp. *oxycedrus*, *J. oxycedrus* subsp. *badia* (H. Gay) Debeaux and *J. oxycedrus* subsp. *macrocarpa* (Sm.) Ball. The subspecies

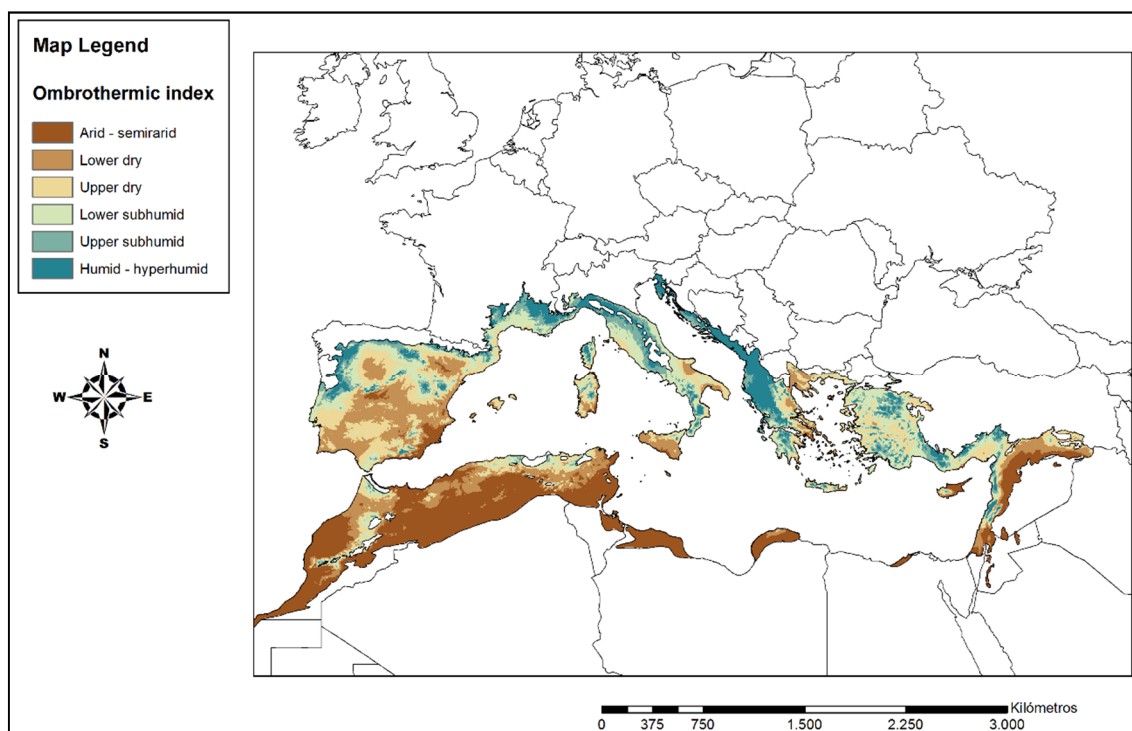
*macrocarpa* is typical of dunes and coastal sand flats and may, occasionally, occupy rocky areas [2], this subspecies is generally a shrub of about 3 m erect and branched with leaves of 20–25 mm × 2–2.5 mm and with a very sharp apex; galls up to 15 mm, somewhat pyriform and purple in color when ripe; however, *J. oxycedrus* subsp. *oxycedrus* and subsp. *badia* are indifferent edaphic, but of dry, sunny and rocky environments.

The communities characterized by this taxon on the Iberian Peninsula are described and included in the alliance *Juniperion turbinatae* Rivas-Martínez 1975 corr. 1987, along with other plant communities presided by *J. navicularis* [synonym of *J. oxycedrus* L. subsp. *transtagana* Franco in Feddes Repert. Spec. Nov. Regni Veg. 68:166 (1963)] and *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman, also typical of psammophilous environments and dunes in coastal zones that fall into the habitat 2250\*. The subspecies *macrocarpa* is an endemic distributed in Spain and in the Balearic Islands, France and Corsica, Italy, Sardinia, Sicily, Morocco, Tunisia, Algeria, Libya, Greece, Crete and Karpathos, the East Aegean Islands, Albania, Bulgaria and Yugoslavia.

The prickly juniper species *J. oxycedrus* L. is widely distributed throughout the Mediterranean area and three subspecies grow in the Iberian Peninsula: *oxycedrus*, *badia* and *macrocarpa* [2]. The subspecies *macrocarpa* and *oxycedrus* extend as far as the Balearic Islands, Corsica, Sardinia and the Italian Peninsula [4], whereas the subsp. *oxycedrus* is found as far as Croatia and Slovenia [5]. At one time the *J. oxycedrus* group included *J. navicularis* (syn.: *Juniperus oxycedrus* subsp. *transtagana*), an endemic taxon of the Iberian Peninsula [6], and *J. deltoides* R.P. Adams [syn.: *J. oxycedrus* L. subsp. *deltoides* (R.P.Adams) N.G. Passal.], which was described as a new species by [7] for Greece. Subsequently, this same author [8] reported the distribution of *J. deltoides* in the central eastern Mediterranean: Italy, Croatia, Greece and Turkey—coexisting in Turkey with *J. polycarpos* K. Koch—and established phytochemical differences with *J. oxycedrus* due to its lower alpha-pinene content and higher limonene content. These data have also been confirmed by [9].

All the taxa in the *J. oxycedrus* group grow in xeric environments, generally in inaccessible places such as limestone or siliceous screes, although isolated individuals may appear in *Quercus rotundifolia* Lam., *Quercus ilex* L. or *Quercus pubescens* s.l. woodlands [10,11]. The plant communities of *Juniperus* are of considerable ecological interest due to the presence of companion endemics, which serves as the justification for their study [12]. They habitually develop communities in small islands that act as species reservoirs as they are not used for either agriculture or livestock farming and thus have not been destroyed by human actions. The places studied are sites of community interest SCI; Habitats Directive 92/43 EEC [13] due to their presence on the vertical walls of habitats such as 8210 “Calcareous rocky slopes with chasmophytic vegetation”, and 8220 “Siliceous rocky slopes with chasmophytic vegetation”, which include many endemic plant associations [14,15] and explain the need to conserve these areas. However, in less steeply sloping rocky areas, the dominant species are *J. oxycedrus* subsp. *badia* and *J. oxycedrus* subsp. *oxycedrus* [12], which characterizes Habitat 5210 “Arborescent matorral with *Juniperus* spp.”. These zones can therefore be classified as hotspots of special interest for conservation. All these associations are included in the Habitats Directive, which confirms the ecological importance of these areas and the need to study them for their subsequent conservation [12,16].

*J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *badia* are found on the Iberian Peninsula on hard acid and basic substrates [12]. The area of distribution of *J. oxycedrus* subsp. *oxycedrus* extends as far as Italy [4], Croatia and Slovenia [5]. It is generally found within *Q. rotundifolia* and *Q. ilex* woodlands and also frequently forms plant communities on screes; as both the subsp. *oxycedrus* and the subsp. *badia* have their optimum development on skeletal-rocky substrates and in bioclimatic environments that oscillate between upper arid-semi-arid and upper dry, but occasionally it can reach the lower subhumid (Figure 1).



**Figure 1.** Optimum areas for the development of *Juniperus oxycedrus* group. Optimal areas range from arid upper to upper dry upper arid-semi-arid and upper dry ombrotype.

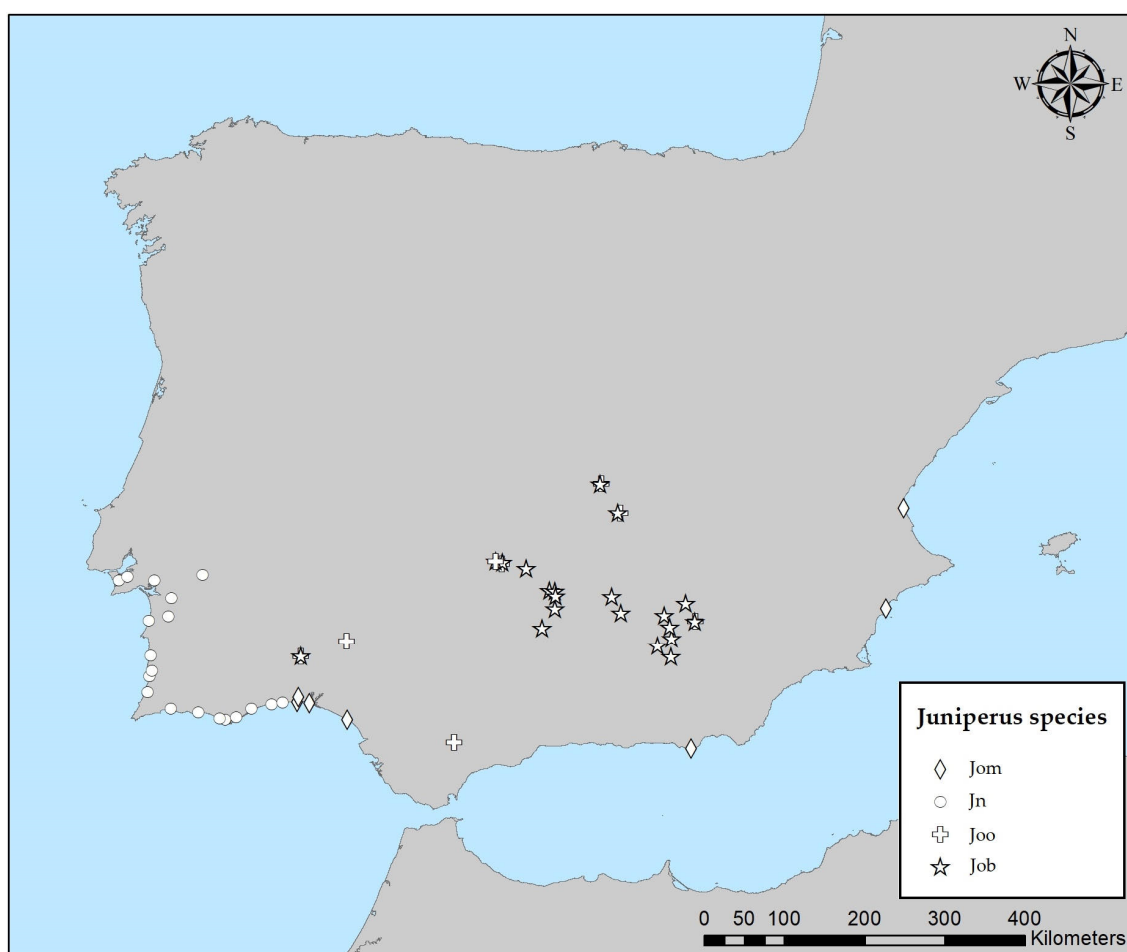
This study, in agreement with others [9,17–20], also confirms that in order to effectively discriminate among species in groups that are difficult to interpret, morphometric, phytochemical and bioclimatic approaches are very useful to clarify the rank to which the taxa belong. The bioclimatic approach is generally also very important for biodiversity conservation, according to [21–27]. As a hypothesis, we maintain that there is a close relation between bioclimatology and ecology and the morphology and phytochemistry of the taxon, and hence with the plant community to which the taxon belongs. The morphometric analyses of leaves, galbula and essential oils reveal no more than genetic expressions, which justifies their use in taxonomy, as we maintained previously with the fractal analysis in *Quercus* leaves [19].

The aim of this work is to clarify the taxonomy, ecology and distribution of the taxa in the *J. oxycedrus* group, which form the communities included in the Habitats Directive 92/43 EEC such as the “Arborescent matorral with *Juniperus* spp.” (5210), in the western Mediterranean.

## 2. Materials and Methods

### 2.1. Sampling Design

We studied the *J. oxycedrus* group and collected specimen and phytosociological samples following previous studies [28,29], inventories of ours and [12,30]. We have used 288 own samples of phytosociological and morphometric character, for the study of leaves and fruits. The work was carried out in the southern half of the Iberian peninsula, to determine the habitat in which these taxa occur. We examined the differences in the ecology, distribution and taxonomy of the taxa in the *J. oxycedrus* group by analyzing their morphological, ecological and phytochemical differences. With these characters, we studied the resemblance between the taxa using a similarity analysis based on the data from Spain (sierra Pandera) [31], Greece and Turkey [32–34] and from SW Portugal (Figure 2), with data extracted from the works of these authors. They used leaves and fruits as analysis material, of the studied localities, and a bioclimatic analysis of said places.



**Figure 2.** Distribution of *Juniperus oxycedrus* group in the study area of the Iberian Peninsula. *Jom* = *J. oxycedrus* subsp. *macrocarpa*, *Jn* = *J. navicularis*, *Joo* = *J. oxycedrus* subsp. *oxycedrus*, *Job* = *J. oxycedrus* subsp. *badia*.

## 2.2. Bioclimatic Analysis

A bioclimatic study was carried out to explain the presence of *Juniperus* species in different rocky substrates. To understand the presence of communities of *Juniperus* in sites dominated by species in the genus *Quercus*, we applied Thornthwaite's index,  $ETP_{monthly} = 16(10.T/I)^a$ , to calculate the potential evapotranspiration and the residual evapotranspiration ( $e$ ) as  $e = 0.2ETP$  by [35]. With these data, we used the ombroedaphoxeric index ( $Ioex$ ) proposed by [12], for this we have used 9 weather stations, in a comparative analysis with the ombro-climatic index  $I_o$  proposed by [36], which justifies the presence of micro-woodlands of *Juniperus* and *Pinus*.

The ombro-thermic index and ombro-climatic index conceptually are synonymous. The word “ombrotype” expresses a category related to the precipitation rate. These three terms are related to the precipitation factor (climate). However, in the calculation of the ombroedaphoxeric index, a second parameter is already involved; soil.

We used phytosociological and bioclimatic studies from different localities (Almadén-Minas, Cabezas Rubias, Aracena, Santiago Pontones, Vadillo Castril, Grazalema, Montoro, Pozoblanco, Villanueva del Arzobispo) [37] to establish the ecology and distribution in Spanish places. For the rest of the Mediterranean, we use data from different authors [12,20,36,38] and the inclusion of *Juniperus* communities in plant associations and in their corresponding habitats [12], as all phytosociological studies specify the ecology and distribution of taxa and plant communities.

Sites behave differently in response to the general climate, the type of substrate and the topography of the terrain. For this reason, areas on rocky crests—although they may be located in rainy environments and surrounded by climactic forests—behave differently

from the areas around them. In these circumstances, islands evolve which may contain edaphoserries, minoriserries and permaserries [39]. All plant communities growing on rocky crests and steeply sloping areas with extreme gradients, among others, are very significantly influenced by the soil, which conditions their existence. All places have a particular type of substrate and an orography that determines whether they have a greater or lesser capacity to retain water. In ideal situations, with good soil texture and structure and with no slopes, the water retention capacity (RC) can be assumed to be at maximum (100%). Otherwise, losses occur due to runoff and drainage, causing the RC to vary. Water is also lost through evapotranspiration (ETP). However, as plants have the capacity to self-regulate their losses, it can be accepted that the residual evapotranspiration  $e = 0.2ETP$ , the value of  $e$  marks the ability of plants to withstand drought. There are therefore two parameters ( $e$  and RC) implicated in the development of a vegetation, which is essentially conditioned by rainfall. The ombro-climatic index  $I_o$ , therefore, does not explain the presence of plant communities influenced by the substrate and, for this reason, we use the ombroedaphoxeric index (Ioex) [12] to explain the presence of communities of *Juniperus* and *Pinus* for territories with a thermotype ranging from the thermo- to the supra-Mediterranean.

The Ioex index is obtained by subtracting the residual evapotranspiration ( $e$ ) from the positive precipitation ( $P_p$ ), the value obtained is divided by the positive temperature ( $T_p$ ) and the result is multiplied by the value taken by RC (0.25, 0.50, 0.75); with the value obtained, the Rivas-Martínez table [40] is applied to obtain the ombic horizons:

$$\text{Ioex} = P_p - e/T_p * RC \quad (1)$$

where  $P_p$  = Positive precipitation (mm);  $T_p$  = Positive temperature ( $^{\circ}\text{C}$ ) [24];  $e$  = residual evapotranspiration whose value is 0.2 ETP [36]; RC = retention capacity in parts per unit, whose values may be 0.25, 0.50, 0.75 and 1; Ioex is the summer ombro-thermal index, calculating Ioex1, Ioex2 and Ioex3 (in function of CR = 0.25, 0.5, and 0.75, respectively); the most representative is Ioex2, which marks the arid character of the locality.

### 2.3. Phytochemical Analysis

For the phytochemical data, we follow the works of [31] and [32] where they describe the material used from Greece and Spain (Jaén herbarium). An Excel table was prepared with the phytochemical data for each taxon and the similarity dendrogram Jaccard was obtained to determine the degree of relation between the taxa. Pearson is used for correlation analysis. The 19 essential oils used are as follows: alpha-pinene, limonene, beta-pinene, myrcene, p-cymene, beta-phellandrene, manoyl oxide, germacrene D, alpha-campholenal, beta-bourbenene, Sesquiterpenes /only for 1 species, gamma-terpinene, terpinen 4-ol, alpha-phellandrene, limonene + beta-phellandrene, alpha-terpinene, terpinolene, alpha-terpinol, Sesquiterpenes only for 1 species.

The number of samples used corresponds to the phytochemical studies of Salido et al. and Adams [32,41]. For statistics, the software PAleontological STatistics (PAST) and Community Analysis Package III were used.

### 2.4. Morphometric Analysis

For the morphometric analysis, we used leaf length and width and galbuli size as characters. For this more than 100 measurements in millimeters (mm) were made for each of the taxa, either from herbarium material or from our own herbalizations, by consulting and reviewing the material from the following herbaria: Herbarium Jaén, Herbarium Algarbe and the Extremadura Centre for Technological Scientific Research (HSS herbarium) (see Supplementary Material), and bibliographic references taken from the Anthos database [42], Flora Ibérica [43], and the database of the flora of Alicante [44]. For the authorship of the taxa we have followed [4,45–51]:

*Juniperus oxycedrus* taxa studied group;  
*Juniperus oxycedrus* L. subsp. *Oxycedrus*;



*J. oxycedrus* L. subsp. *badia* (H. Gay) Debeaux; Syn. *J. oxycedrus* L. var. *badia* H. Gay;  
*J. oxycedrus* L. subsp. *macrocarpa* (Sm.) Ball; Syn. *J. macrocarpa* Sm.;  
*J. navicularis* Gand; Syn. *J. oxycedrus* L. subsp. *transtagana* Franco;

### 3. Results and Discussion

#### 3.1. Bioclimatic Analysis

Table 1 shows the values of Pp (Positive Precipitation), Tp (Positive Temperature) and Io (Ombro-climatic Index) according to the criterion established by Rivas-Martínez. Thornthwaite's formula,  $ETP_{monthly} = 16(10.T/I)^a$ , is applied to obtain the value of ETP (potential evapotranspiration), where T is the mean monthly temperature, I is the annual heat index and a parameter that depends on the values taken by I. [12].

Applying the formula  $I_{oex}$  for the assumptions that RC is 0.25, 0.50, 0.75, we obtain three values of which the most representative is  $I_{oex2}$ . Table 1 shows the equivalence values so that although the territorial bioclimate allows the existence of climactic forests in wild areas with RC = 50%, the humid ombrotype becomes dry or subhumid depending on whether the value of RC = 25% or 50%. The subhumid becomes dry and the dry becomes semiarid or arid. Therefore, in areas with  $I_o > 8$  we obtain  $I_{oex2}$  values of 3.86 and 4.94, which is the equivalent to subhumid. This explains the fact that, in rocky areas, there is an edaphoxerophilous community of *Quercus faginea* s.l. or *Abies pinsapo* Boiss., as occurs in Grazalema (Cadiz); or that a value of  $I_{oex1} = 2.47$  is obtained in the case that RC = 25%. In this situation, there is a presence of an edaphoxerophilous community of *Quercus rotundifolia* in Cazorla (Jaén) and in Grazalema (Cadiz). When the underlying ombrotype is subhumid, the equivalence value of  $I_{oex2}$  corresponds to dry; and a situation that initially has a dry  $I_o$  gives semiarid and even arid values of  $I_{oex2}$ , if we start from a horizon that is lower than dry. This does not allow the development of *Quercus* tree species but does allow the development of the *Juniperus* and *Pinus* genera. The value of  $I_{oex}$  is affected by climate change, as evidenced by Del Río et al. [38], who report a heterogeneous trend in terms of annual rainfall redistribution, with a decline in most of the mountainous areas of Grazalema, Ronda, Cazorla, Segura, Sierra Nevada and a large part of the Sierra Morena. However, these authors have detected an increase in rainfall on the Andalusian coast and particularly in Almería. This affects the forest stands and, in conjunction with human activity [50], favors a redistribution of the current forests with a decline in *Quercus* woodlands and an increase in the micro-woodlands of *Juniperus*.

**Table 1.** Comparative value of  $I_o$  and  $I_{oex}$  indices in some localities in the southern Iberian Peninsula.  $P_p$  = Positive precipitation (mm),  $T_p$  = Positive temperature ( $^{\circ}C$ ),  $I_o$  = Ombro-climatic index, ETP = Potential evapotranspiration (mm),  $e$  = residual evapotranspiration,  $I_{oex1}$ ,  $I_{oex2}$  and  $I_{oex3}$  = Summer ombro-climatic indices (CR = 0.25, 0.5 and 0.75, respectively). Ombro-climatic behavior of the locality is synonymous with ombrotype applied to a specific locality.

Weather Station. 25–30 Years	$P_p$ (mm)	$T_p$ ( $^{\circ}C$ )	$I_o$	Ombrotype	ETP (mm)	$e$ (mm)	$I_{oex1}$	$I_{oex2}$	$I_{oex3}$	Ombro-Climatic Behaviour of the Locality
Almadén-Minas	625.20	194.40	3.20	dry	808.54	161.70	0.59	1.19	1.78	semiarid
Cabezas Rubias	993.40	177.60	5.60	subhumid	702.14	140.42	1.20	2.40	3.60	dry
Aracena (H)	1025.80	175.20	5.90	subhumid	703.46	140.69	1.26	2.52	3.78	dry
Santiago Pontones	1148.70	164.40	7.00	subhumid	675.23	135.04	1.54	3.08	4.62	dry
Vadillo Castril	1182.20	140.40	8.40	humid	488.88	97.72	1.93	3.86	5.79	subhumid
Grazalema	1962.20	183.60	11.00	humid	726.22	145.24	2.47	4.94	7.42	subhumid
Montoro	522.40	210.00	2.50	dry	903.15	180.63	0.40	0.81	1.22	arid
Pozoblanco	514.40	193.20	2.70	dry	805.45	161.09	0.45	0.91	1.37	arid
Villanueva del Arzobispo	698.20	196.80	3.50	dry	915.70	183.14	0.65	1.30	1.96	semiarid

### 3.2. Phytochemical Analysis

Table 2 shows that the alpha-pinene is common to the four taxa and p-cymene to *J. oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *macrocarpa* and *J. navicularis* (Table 2). This is justification for including them all in the *J. oxycedrus* group. Moreover, there are certain morphological and ecological similarities between *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *badia* and between *J. oxycedrus* subsp. *macrocarpa* and *J. navicularis*, according to the presence of certain oils. The component myrcene is exclusive to the subspecies *oxycedrus*, whereas the exclusive compounds in the subspecies *badia* are: germacrene D, alpha-campholenal, beta bourbenene and nine sesquiterpenes. The subspecies *macrocarpa* does not have any of its own essential oils as it shares gamma-terpinene and terpinen 4-ol with *J. navicularis*, while the exclusive chemical compounds in *J. navicularis* are alpha-phellandrene, alpha-terpinene, terpinolene, alpha terpinol and ten sesquiterpenes. The species *J. navicularis* presents different sesquiterpenes from those of *J. oxycedrus* subsp. *badia*.

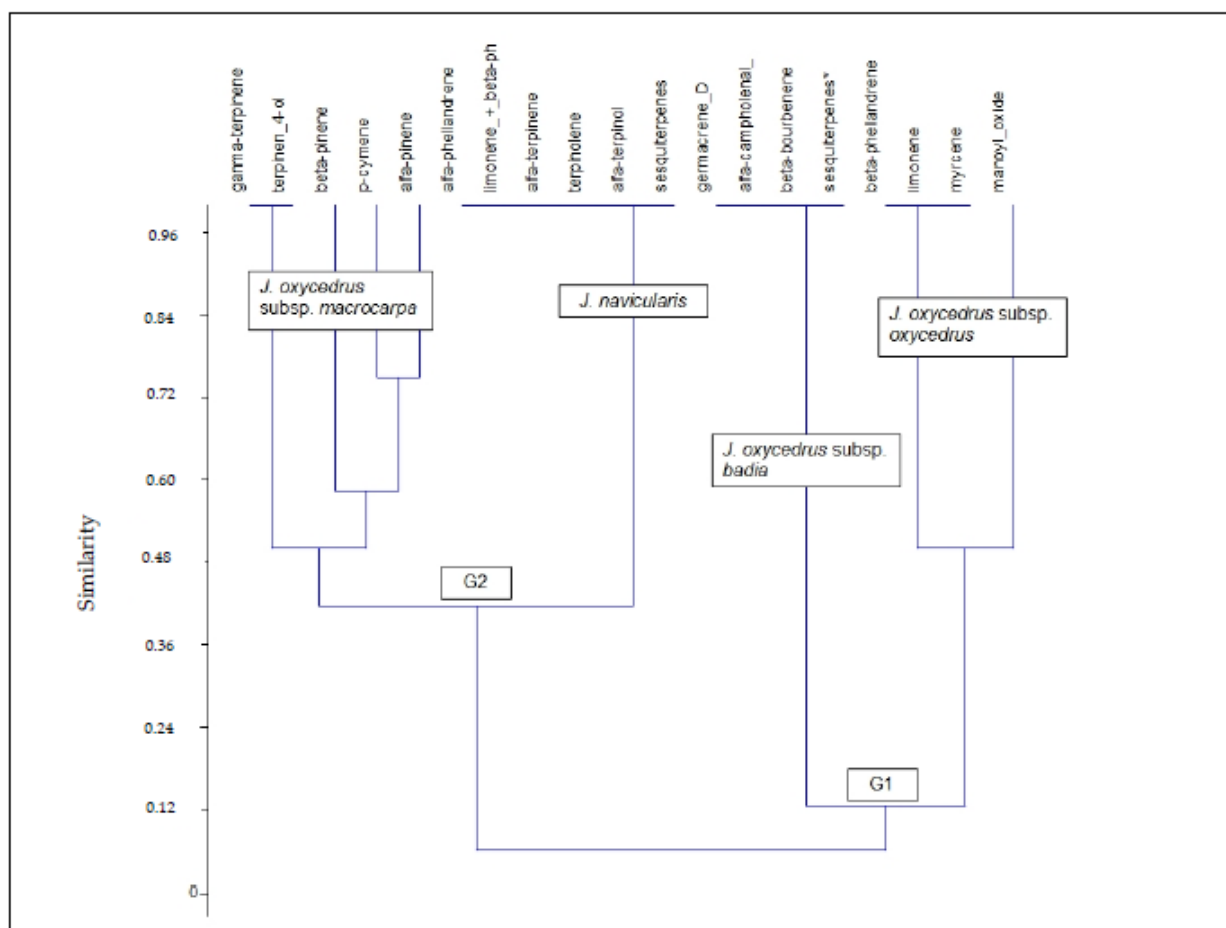
**Table 2.** Essential oil content in four *Juniperus* species. Units of measure in gr, values in %, from [31,32].

	Taxa			
	<i>J. oxycedrus</i> subsp. <i>oxycedrus</i>	<i>J. oxycedrus</i> subsp. <i>badia</i>	<i>J. oxycedrus</i> subsp. <i>macrocarpa</i>	<i>J. navicularis</i>
<b>Essential oils</b>				
alpha-pinene	34	0.1	0.1	30.8
limonene	16.25	0	0	0
beta-pinene	0.1	0	0	3.3
myrcene	0.1	0	0	0
p-cymene	0.1	0	0.1	3
beta-phellandrene	0.1	0	0	0
manoyl oxide	0.1	0.1	0	0
germacrene D	0	0.1	0	0
alpha-campholenal	0	0.1	0	0
beta-bourbenene	0	0.1	0	0
Sesquiterpenes/only for 1 species	0	0.1	0	0
gamma-terpinene	0	0	0.1	0.5
terpinen 4-ol	0	0	0.1	0.9
alpha-phellandrene	0	0	0	11.1
limonene + beta-phellandrene	0	0	0	27.2
alpha-terpinene	0	0	0	0.5
terpinolene	0	0	0	3.4
alpha-terpinol	0	0	0	1.1
Sesquiterpenes only for 1 species	0	0	0	0.1

As specified by [31] and [32], there is a clear phytochemical differentiation between the three subspecies of *J. oxycedrus* (*J. oxycedrus* subsp. *oxycedrus*, subsp. *badia* and subsp. *macrocarpa*). Subsequently [8] clarifies the phytochemical differences between *J. oxycedrus* and *J. deltoides*, a species described by this author [7] in Greek territories. This taxon has a low content in alpha-pinene and a high content in limonene, which, among other morphological differences, justifies the rank of species for this taxon. There are also substantial differences in the essential oils of *J. navicularis* Gand. compared to the rest [33].



The Pearson correlation analysis (Table 3) gives values of  $r = 1$  or near 1 for group G2 (*J. oxycedrus* subsp. *macrocarpa* and *J. navicularis*). In the case of group G1 (*J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *badia*) the values are close or equal to 1; however, the relationship between both groups is low owing to the phytochemical differences between them. In Jaccard's similarity analysis in Figure 3, two clearly differentiated groups (G1 and G2), which is due to the concentrations and different chemical components they present.



**Figure 3.** Similarity analysis between the taxa *Juniperus oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *badia*, *J. oxycedrus* subsp. *macrocarpa* and *J. navicularis*.

**Table 3.** r-Pearson. The values in bold are different from 0 with a level of significance of alpha = 0.05. Types of oils considered: 1. Alpha-pinene, 2. Limonene, 3. Beta-pinene, 4. Myrcene, 5. P-cymene, 6. Beta-phellandrene, 7. Monoyl oxide, 8. Germacrene D, 9. Alpha-campholenal, 10. Beta-bourbenene, 11. Sesquiterpenes exclusives, 12. Gamma-terpinene, 13. Terpinen 4-ol, 14 Alpha-phellandrene, 15. Limonene + beta-phellandrene, 16. Alpha-terpinene, 17. Terpinolene, 18. Alpha-terpinol, 19. Sesquiterpenes.

Essential Oils																		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<b>1</b>	0.63	0.543	0.633	0.532	0.633	0.049	−0.58	−0.58	−0.58	−0.58	0.424	0.47	0.519	0.519	0.519	0.519	0.519	0.519
-	<b>1</b>	−0.31	<b>1</b>	−0.32	<b>1</b>	0.577	−0.33	−0.33	−0.33	−0.33	−0.42	−0.38	−0.33	−0.33	−0.33	−0.33	−0.33	−0.33
-	-	<b>1</b>	−0.31	<b>1</b>	−0.31	−0.57	−0.35	−0.35	−0.35	−0.35	<b>0.98</b>	<b>0.99</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>
-	-	-	<b>1</b>	−0.32	<b>1</b>	0.577	−0.33	−0.33	−0.33	−0.33	−0.42	−0.38	−0.33	−0.33	−0.33	−0.33	−0.33	−0.33
-	-	-	-	<b>1</b>	−0.32	−0.59	−0.36	−0.36	−0.36	−0.36	<b>0.98</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>
-	-	-	-	-	<b>1</b>	0.577	−0.33	−0.33	−0.33	−0.33	−0.42	−0.38	−0.33	−0.33	−0.33	−0.33	−0.33	−0.33
-	-	-	-	-	-	<b>1</b>	0.577	0.577	0.577	0.577	−0.73	−0.66	−0.58	−0.58	−0.58	−0.58	−0.58	−0.58
-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	−0.42	−0.38	−0.33	−0.33	−0.33	−0.33	−0.33	−0.33
-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>1</b>	−0.42	−0.38	−0.33	−0.33	−0.33	−0.33	−0.33	−0.33
-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	−0.42	−0.38	−0.33	−0.33	−0.33	−0.33	−0.33	−0.33
-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>0.98</b>	<b>0.98</b>	<b>0.98</b>	<b>0.98</b>	<b>0.98</b>	<b>0.98</b>
-	-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>0.99</b>	<b>0.99</b>	<b>0.99</b>	<b>0.99</b>	<b>0.99</b>	<b>0.99</b>
-	-	-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>
-	-	-	-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>1</b>
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>

### 3.3. Morphometric Analysis

The main differences between *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *badia* are based on their physiognomy and the size of their mature fruits [2]. Whereas the subspecies *oxycedrus* tends to occur as a shrub, the subspecies *badia* is a tree of considerable height and with a pyramidal form. The size of the mature galbuli in the subspecies *oxycedrus* does not generally exceed 1 cm, while in the subspecies *badia* it is over 1 cm. The leaves of subspecies *oxycedrus* have a width of 1–1.5 mm while those of subsp. *badia* are 1.2–2 mm. Coincidentally these subspecies are frequently found coexisting in similar biotopes, which has led to frequent confusion by several authors. The subsp. *macrocarpa* has galbuli of 1.2–1.5 cm with a purplish chestnut color when ripe; this is an erect tree, rarely prostrate, growing up to 3 m. In contrast *J. navicularis* has a similar ecology to subsp. *macrocarpa* but grows to a maximum height of 2 m; its leaves are 1–1.5 mm wide and its galbuli are between 0.7–1 cm (Table 4).

**Table 4.** Morphometric comparison in the taxa of *Juniperus oxycedrus* group. The samples analyzed correspond to Portugal for *J. navicularis*, Southern Spain for *J. oxycedrus* subsp. *macrocarpa*, South and Central Spain for *J. oxycedrus* subsp. *oxycedrus*, and subsp. *badia* (Supplementary material). Millimeters (mm) have been used as units of measurement for leaves and galbuli and meters for the height of the tree or shrub. A1, B1, C1, D1 bibliographic measures. A2, B2, C2, D2 own measurements. A. *J. oxycedrus* subsp. *oxycedrus*; B. *J. oxycedrus* subsp. *badia*; C. *J. oxycedrus* subsp. *macrocarpa*; D. *J. navicularis*.

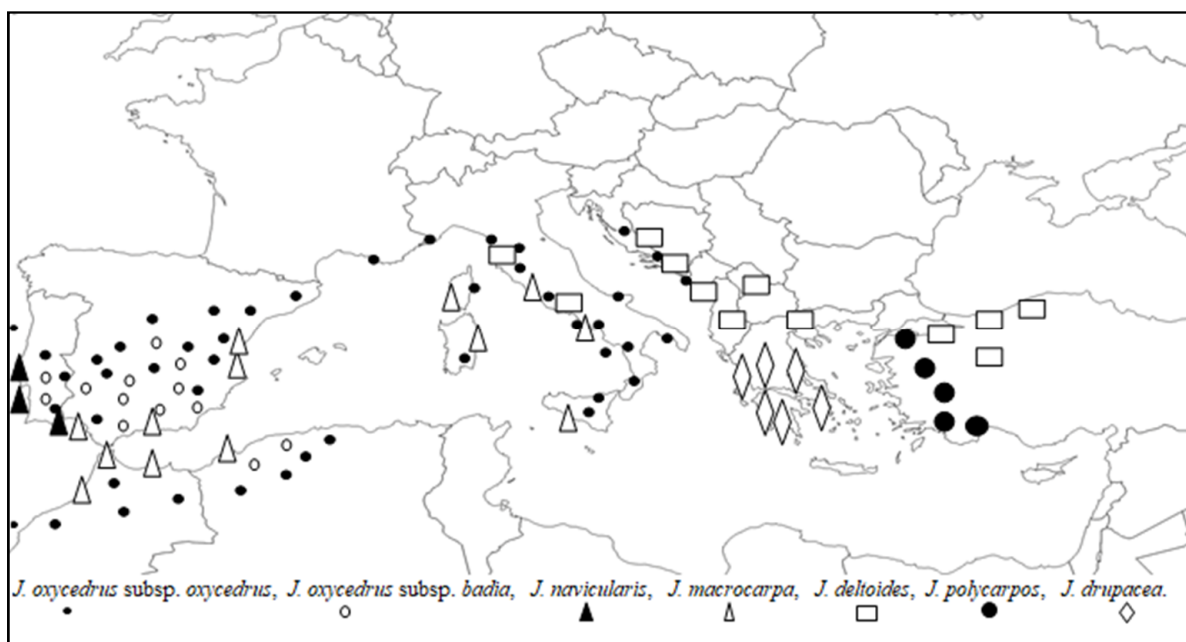
	A1	A2	B1	B2	C1	C2	D1	D2
leaf length (mm)	8–15(25)	12–18	12–20	8–20	20–25	12–20	4–12	6–10
leaf width (mm)	1–1.5	<1.5	1.2–2	>1.5	2–2.5	<3	1–1.5	<1.5
galbuli (mm)	8–10	6–12	10–13	>10	12–15	12–15	7–10	5–10
tree habit (m)	scrub < 4	scrub < 6	tree < 15	tree 7–12	scrub < 3	scrub < 5	scrub < 2	scrub 1–2

Linnaeus did not describe *J. oxycedrus* in the first part of his work *Species Plantarum*, but merely noted the existing synonym [52]. Theoretically, the authorship of *J. oxycedrus* L. should be attributed to Clusius, who described it: “Nusquam autem majorem vidisse memini, quam supra Segoviam et Guadarrama, itinere Madritiano, ubi magnarum arborum interdum altitudinem”. He also says of the fruit “fructum initio viridem... postremo, cum maturuit punicea coloris, Juniperi fructu multo majorem ut interdum avellanam magnitudine aequet” [53]. This description is specifically included in the work of [45]. Clusius’ description, in which he states literally that it has a “thick pruinose red fruit”, corresponds to *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux [Basion. *Juniperus oxycedrus* var. *badia* H. Gay in Assoc. Franç. Avancem. Sci. Compt. Rend. 1889:501 (1889)], included in [2]. The lack of typification of the specimen described by Clusius is justification for these facts and supports the valid acceptance of *Juniperus oxycedrus* subsp. *badia* (ICN article 41). Due to the high number of morphological and ecological differences and differences in essential oils between subsp. *macrocarpa*, subsp. *oxycedrus* and subsp. *badia*, and to its similarity with *J. navicularis*, the subspecies *macrocarpa* can be proposed to be subordinated to *J. navicularis* as *J. navicularis* Gand. subsp. *macrocarpa* (Sm.) Ball; however, the molecular study by [41] establishes major molecular differences between the four taxa *J. oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *badia*, *J. oxycedrus* subsp. *macrocarpa* and *J. navicularis*, and based on this he raises them to the rank of species. The phytochemical differences between groups G1 and G2 are very clear, so the species rank could be accepted for the four taxa. However, in the case of their distribution, the populations of *J. oxycedrus* subsp. *macrocarpa* and *J. navicularis* are separated despite having similar ecologies, while this is not the case of *J. oxycedrus* subsp. *oxycedrus* and subsp. *badia*. Therefore, it is logical to accept *J. macrocarpa* and *J. navicularis* as species and to maintain *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* L. subsp. *badia* (H. Gay) Debeaux with the rank of subspecies.

There are also some doubts about the presence of *J. oxycedrus* subsp. *badia* in the African continent. Some authors such as [47] do not recognize this taxon in northern Africa,

although [54] for this area reports *J. oxycedrus* subsp. *badia* [sub *J. rufescens* (Link) Deb. f. *badia* H. Gay)] and subsp. *macrocarpa*.

Bolòs & Vigo [55] include the var. *laguna* Pau ex Bolòs et Vigo in *J. oxycedrus* subsp. *oxycedrus*, as it has the same characters as the subspecies *badia*. Recently, based on the work of [56,57], a new combination of *Juniperus oxycedrus* subsp. *lagunae* was formulated. This all serves to highlight the complexity of this taxon, whose distribution area is insufficiently known. However, its presence is very evident in the center and south of the Iberian Peninsula, where it grows in formations with a broad extension, generally on scree and biotopes with shallow soils. In these habitats, holm oaks (*Quercus rotundifolia*) cease to be dominant or simply cannot exist due to the absence of the necessary ecological and/or soil conditions for these taxa to develop [30] (Figure 4).



**Figure 4.** Distribution of the *Juniperus oxycedrus* group in the Mediterranean basin: Coexistence of populations of *J. oxycedrus* subsp. *oxycedrus* and subsp. *badia* in the Iberian Peninsula.

The areas dominated by the species of juniper are currently undergoing a process of expansion due to the increase in areas of scree and rocky areas in general. This phenomenon causes an increase in dry areas and a decrease in rainy areas, thus expanding the potential areas that can act as a refuge for endemic species [58]. A good approach for planning the re-naturalization of habitats with *Juniperus* species is to use autochthonous species after fire or other environmental events, which is also effective for preserving the local genetic resources and avoiding genetic pollution [26,59]. Another good approach is phytotoponym analysis in order to gain a better understanding of their potential areas of growth, as proposed by [60].

In regard to the bioclimatology of the *Juniperus oxycedrus* group, its thermotype ranges between the thermo- and meso-Mediterranean, occasionally reaching the supra-Mediterranean; the ombrotype is between arid and dry and, in rocky areas, may reach the subhumid. These special bioclimatic and ecological characteristics account for the distribution of these *Juniperus* taxa, and allow a correlation to be established with their phytochemistry, which explains the differences between the type and concentration of their essential oils.

The bioclimatic and ecological differences between the taxa can be seen from their dissimilar morphological and phytochemical features. They differ significantly in the length and width of the leaves and galbuli, and even more so in their phytochemistry. The

similarity analysis connects *J. oxycedrus* subsp. *macrocarpa* with *J. navicularis*, two taxa that effectively have their ecological optimum in sandy thermo-Mediterranean areas; whereas *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *badia* show a greater degree of similarity and have their optimum in more continental meso-Mediterranean environments.

Vasic and Dubak [1] discuss the anatomical characters (epidermis) of *J. oxycedrus* at various altitudes in the mountains in Serbia. Although these authors report that the morphological characters may vary somewhat, this is not the case as differences in the essential oils have been observed in the present study. In studies on the biogeography and genetic relations of *J. oxycedrus* in the Mediterranean, including Macaronesian regions, Boratyński et al. [61] demonstrate the close relation between *J. navicularis*, *J. oxycedrus* subsp. *macrocarpa* and *J. brevifolia* (Seub.) Antoine de Azores, and describe in detail the genetic separation between *Juniperus* from the eastern Mediterranean and *Juniperus* from the western Mediterranean—which currently includes Macaronesia. As in our study, their morphological analysis confirmed the separation between *J. navicularis* and *J. oxycedrus* subsp. *macrocarpa* and *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *badia*. Thashami & Aggag [62] arrived at similar conclusions when they separated *J. oxycedrus* subsp. *oxycedrus* from *J. oxycedrus* subsp. *macrocarpa* through morphological and genetic analyses. Juan et al. [63] studied *J. oxycedrus* subsp. *macrocarpa* on the Atlantic and eastern coasts of the Iberian Peninsula and the Balearic Islands, from the genetic and phylogeographic point of view, but did not establish a relation with the other taxa from the *oxycedrus* group on the Iberian Peninsula.

Finally, from the phytosociological standpoint, in vegetation studies the subspecies *badia* (H. Gay) Debeaux and the subspecies *lagunae* (Pau ex Vicioso) Rivas-Martínez have been used indistinctly because of accumulated and persistent errors [30]. The name *J. oxycedrus* L. subsp. *lagunae* (Pau ex Vicioso) Rivas-Martínez is invalid and based on the iconography of Laguna by Pau [*J. oxycedrus* L. subsp. *lagunae* (Pau ex C. Vicioso) Rivas Mart. Itinera Geobot. 15(2): 703 (2002); nom. inval.], as the name of the basionym is not validly published (ICN article 7). The correct name is therefore *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux. Based on these facts, the various authors of plant associations must rectify their names.

#### 4. Conclusions

The similarity study based on the quantity, type of essential oils, and different bioclimatic distribution could justify maintaining the rank of species for *J. macrocarpa* Sm. and *J. navicularis* Gand. Instead, the distribution and co-existence of the two remaining taxa, in spite of their phytochemical differences, do not warrant the rank of species and we thus maintain their rank as subspecies: *J. oxycedrus* L. subsp. *oxycedrus* and *J. oxycedrus* L. subsp. *badia* (H. Gay) Debeaux. Due to the high number of morphological and ecological differences and differences in essential oils between subsp. *macrocarpa*, subsp. *oxycedrus* and subsp. *badia*, and to the similarity of subsp. *macrocarpa* with *J. navicularis*, this subspecies can be proposed to be subordinated to *J. navicularis* as *J. navicularis* Gand. subsp. *macrocarpa* (Sm.) Ball. Being of special reference for the differentiation between the types of junipers, the morphometric character of leaves and galbulas, types and content in essential oils, and the exclusive thermo-Mediterranean character of *J. navicularis* and *J. navicularis* Gand. subsp. *macrocarpa*.

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