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# Tree-ring chronologies, stable strontium isotopes and biochemical compounds: Towards reference datasets to provenance Iberian shipwreck timbers

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## ABSTRACT

Studies on the provenance of wood for shipbuilding contribute widely to the fields of archaeology, anthropology, environmental history, cultural geography, and palaeoclimatology. The development of reference datasets to determine the date and provenance of shipwreck timbers is therefore a paramount undertaking. Here we compile and present recent advances in the development of tree-ring chronologies, stable strontium isotope ratios and chemical biomarkers aimed to determine the date and provenance of Iberian shipwreck timbers. A set of oak and pine tree-ring chronologies have been developed from living trees covering the past 500 and 800 years, respectively, and have served to confirm the provenance of the wood used in an 18th-century Spanish ship of the Royal Navy. Stable strontium isotopic signatures have been obtained from soil and living trees at 26 sites throughout the Iberian Peninsula, providing a climate-independent geochemical network to source the origin of historic timbers. However, retrieving the original isotopic signature from waterlogged samples remains unsuccessful, stressing the need to develop effective protocols to separate the seawater signal from the original strontium isotope ratios in the wood. Analyses of organic compounds in wood of living trees have proven suitable to discriminate species and provenances, but results on shipwreck timbers are inconclusive and should be further explored. Our regional approach has the potential to be expanded to other areas and archaeological timbers from different periods throughout the Anthropocene. We highlight the strengths and weaknesses of the techniques presented when applied to waterlogged wood, propose GIS tools to interpret and visualize combined results, and stress the need to expand these type of reference datasets to allow for multiproxy dendroprovenancing approaches.

## 1. Introduction

In disciplines related to cultural heritage studies and archaeology,

determining the date and provenance of structures and artefacts is paramount to establish their specific historical and socio-cultural contexts. In the case of maritime archaeology, timber elements that

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compose shipwrecks, ancient harbours and other coastal or riverine structures of all historical periods provide a wealth of information that can be retrieved through archaeometric techniques and biochemical analyses. Because tree species register environmental information about the place where they grow (e.g. climatic conditions registered in annual growth ring-patterns and anatomical features; soil composition reflected in organic and inorganic chemical compounds in the wood), novel methods are being sought to use this information to pinpoint the origin of timbers (Dormontt et al., 2015). Inferences about ancient craftsmanship, human-environment interactions and paleoclimate can therefore be made for specific territories and time periods.

While commonly used for determining the age of wooden artefacts, dendrochronology also serves to identify the geographical source of the wood (Bridge, 2012). There are, however, inherent limitations that can hinder its application in certain circumstances: for example, timber samples originating from areas without tree-ring reference datasets can be neither dated nor provenanced, and tree-ring patterns that retain weak climatic signals (i.e. patterns with complacent rings) may prove insufficient for strong statistical correlations with reference data.

These constraints to dendroprovenancing historic timber by conventional dendrochronology have long been convergent in the Iberian Peninsula, where tree-ring studies had typically focussed on ecological and/or climatological questions (Domínguez-Delmás et al., 2015; Gazol et al., 2018). Long-span tree-ring chronologies suitable for dating and provenancing historical wood were scarce (Richter and Eckstein, 1986; Susperregi, 2007; Domínguez-Delmás et al., 2013), and some of the forests that supplied timber resources for shipbuilding during the Age of Discovery and European expansion (c. 1500–1800 CE) are currently depleted or mere relics of the woodlands they once were (Domínguez-Delmás et al., 2015). Therefore, together with the development of long-span tree-ring chronologies in the vicinity of areas formerly exploited for shipbuilding, other empirical techniques have been explored to determine the origin of Iberian timbers from archaeological contexts. These methods include the development of earlywood-vessel chronologies of oak (*Quercus robur*, *Q. petraea*, *Q. faginea* and *Q. pyrenaica*) (Akhmetzyanov et al., 2019) and latewood density chronologies of pine species (*Pinus sylvestris* and *P. nigra*) (Akhmetzyanov et al., 2020), the analysis of strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) as provenance markers (Hajj et al., 2017), and the identification of tree species using Fourier-transform infrared spectroscopy (FTIR) and Pyrolysis-Gas Chromatography-Mass Spectrometry (PGC-MS) (Traoré et al., 2018a, 2018b). The efficacy of some of these methods (namely, traditional dendroprovenancing with tree-ring chronologies, stable Sr isotope ratios, and identification and quantification of organic compounds by FTIR and PGC-MS) has been tested with ship timbers retrieved from several suspected Iberian shipwrecks (i.e. wrecks from ships thought to have been built in Atlantic-Iberian shipyards). Here we compile the results of those tests, describing the potential and limitations of these methods to establish the provenance of waterlogged wood. We also propose a way to integrate the results of these scientific methods using a database linked to a geographic information system (GIS).

## 2. Departing hypothesis and sampling strategy

In different periods and geographical areas, timber for shipbuilding could have been sourced from woodlands relatively close to the shipyards, as well as from forests located in more distant territories (Albion, 1926; Meiggs, 1982; De Vries and Van der Woude, 1997). By establishing the provenance of the wood used in ship timbers, inferences can be made regarding former forest management practices at specific sites, timber-trade networks, landscape changes, environmental conditions prevailing at the sites where the trees grow, and how all these interactions evolved through time. In this way, studies on the provenance of wood for shipbuilding contribute widely to the fields of archaeology, anthropology, environmental history, cultural geography, and palaeoclimatology (Rich, 2017).

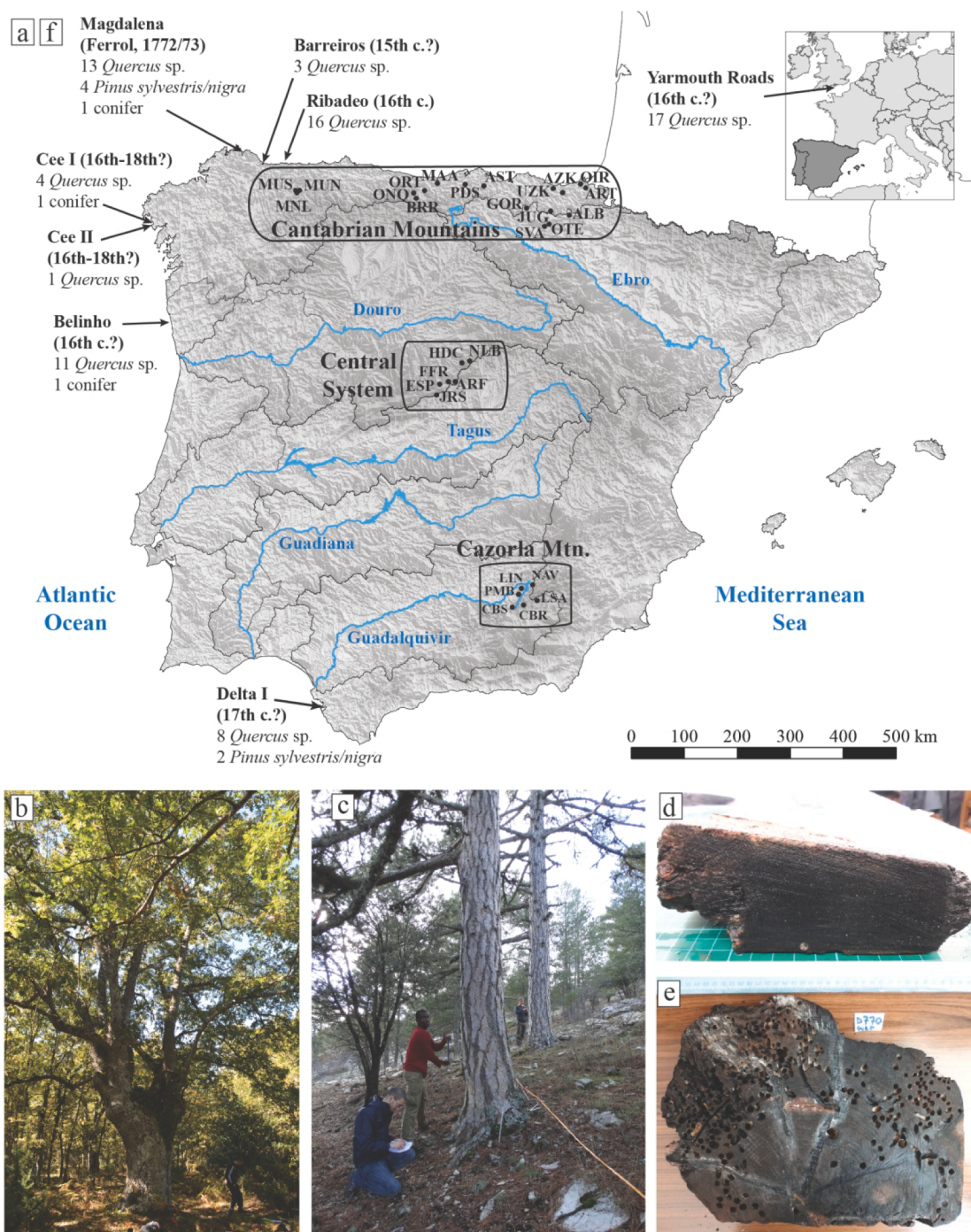
In 2014, the Marie Skłodowska-Curie Innovative Training Network ForSEADiscovery project ([www.forseadiscovery.eu](http://www.forseadiscovery.eu)) was launched to investigate the supply of timber for Iberian empires in the Early Modern Period (1500–1800), especially in relation to naval shipbuilding. The arrival of Christopher Columbus to the Americas in 1492 set in motion the era of European expansion, globalisation and colonisation, in which Spanish and Portuguese seafarers played a paramount role (Castro, 2005). Ship designs evolved to accommodate higher tonnage and cope with oceanic voyages (Edwards, 1992; Hancock, 2017). The chronology of changes to ship designs is still uncertain, as is the role played in those changes by the availability of tools, manpower, and raw materials (Edwards, 1992; Loewen, 2001). To help develop the chronology of those changes and its relationship to craftsmanship and materials, the ForSEADiscovery project set up a concerted multidisciplinary investigation into the primary raw material for Iberian shipbuilding: that is, timber.

The construction of Iberian oceangoing vessels such as carracks, galleons and caravels demanded timber in amounts beyond what local forests could provide, so the state gradually increased control over the woodlands to ensure a steady supply for the navy (Loewen, 2000; Wing, 2015). Shortly thereafter, timber from northern Europe began being imported into the Iberian Peninsula for shipbuilding (De Aranda y Antón, 1990; Jiménez Montes, 2020). Consequently, a departing hypothesis of the ForSEADiscovery project was that Iberian empires used local and imported timber to build their ships, but they did not export wood from Iberian forests to foreign nations. Local timber was too valuable a resource to export. Therefore, if we could identify numerous timbers from a shipwreck as derived from trees grown in the Iberian Peninsula, it would be strong evidence that the ship was built at an Iberian shipyard. To address the question of timber provenance, a multidisciplinary approach was devised, whereby history and nautical archaeology were combined with disciplines from the natural sciences (dendrochronology, organic and inorganic chemistry, wood anatomy, and DNA) (Crespo Solana and Nayling, 2015). Data obtained would not only provide valuable cultural and environmental information on the deforestation of the Iberian Peninsula, but they would also augment our collective knowledge of ship construction techniques, which in turn would contribute to the longstanding discussion of construction features unique to the Atlantic-Iberian shipbuilding tradition (Loewen, 1998; Oertling, 2001; Castro, 2008; Loureiro, 2012).

To develop and test our new datasets for the Iberian Peninsula (tree-ring based chronologies, vessel and latewood density chronologies, site-specific signatures of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, and organic compounds that determine species-level differences), a selection of sampling sites was made by considering locations identified in historical documents as timber sources for shipbuilding during the Early Modern Period. We selected two main geographical areas based on De Aranda y Antón (1990): the Cantabrian Mountains in northern Spain, and the Cazorla Mountain Range in southern Spain (Fig. 1a). We also selected several sites in central Spain (Central System) to ascertain whether timber from that area was floated down the Douro and Tagus rivers towards western Spain or Portugal. Within those areas, specific sites were chosen based on their logging history and their waterway connections to coastal shipyards (Table 1). Some of these sites lacked old trees of the target species, so we also included nearby areas where older trees could be found. Targeted species comprised those traditionally used in structural elements of ships, mainly oaks (*Quercus* sp.) and pines (*Pinus* sp.) (De Aranda y Antón, 1990; Goodman, 2003; Wing, 2015). In the Iberian Peninsula, the genus *Quercus* comprises a range of evergreen and deciduous species, but we focused on the predominant species in northern Spain: *Quercus robur*, *Q. petraea*, *Q. faginea*, and *Q. pyrenaica*, all of them deciduous oaks. The pine species selected for this study were *Pinus nigra* and *P. sylvestris*, which are predominant in the south (Cazorla Mountains) and Central System respectively.

At each site, we collected samples for tree-ring analysis using standard increment borers of 5 mm diameter and lengths ranging from 30 to





**Fig. 1.** a) Geographical map of major Iberian watersheds indicating the main areas and sites selected to develop the reference datasets of tree-ring chronologies, Sr isotopes signatures and organic compounds (full site names are listed in Table 1); b) sampling site (oaks) in the northeast of Spain (Basque Country); c) sampling pine (*P. nigra*) in the south (Cazorla Mountains); d) sample from a starboard radial oak plank (*Quercus* subg. *Quercus*) with sapwood from the Yarmouth Roads shipwreck; e) keel cross-section of oak (*Quercus* subg. *Quercus*) from the Delta I shipwreck; f) names and locations of the shipwrecks mentioned in the text, listing the number of samples that were analysed by dendrochronology within the ForSEADiscovery project and their species when known (for a full list of sampled elements per shipwreck please refer to Table S1 in Supplementary Material).

60 cm depending on the diameter of the tree (Fig. 1b, c). Six to 85 trees were sampled per site, with a minimum of two radii per tree for dendrochronological analyses. From five trees at selected sites, we collected two additional samples (Table 1): one for strontium isotopic analysis, and another for analyses of organic compounds.

A second dataset consisted of samples of archaeological timbers from selected shipwrecks studied during the ForSEADiscovery project (Fig. 1f; Table S1 in Supplementary Material). Three of those wrecks are known

or thought to have been ships constructed in Iberian shipyards according to historical records and/or archaeological research: *La Santa Maria Magdalena* (18th-century frigate, Viveiro, Spain) (Trindade et al., 2020); the Delta I (17th-century merchant vessel, Cadiz, Spain) (Bernáldez Sánchez et al., 2013; Guerrero López and Alzaga García, 2013; Higuera-Milena Castellano et al., 2013; Higuera-Milena Castellano and Gallardo Abarzuza, 2016); and a shipwreck at Yarmouth Roads (Isle of Wight, UK), which may be identified as the *Santa Lucia* (a



**Table 1**

Geographic information of the sampling sites (living trees), including species present, and datasets developed.

Region	Site	CODE	Latitude	Longitude	Elevationm a.s.l.	Aspect	Species present	Dendro-data	Sr isotopes	Organic compounds
North	Artikutza	ART	43.22412	−1.78352	440	E	<i>Q. robur</i>	No	Yes	Yes
North	Astrana	AST	43.21178	−3.55616	840	W	<i>Q. pyrenaica</i>	No	Yes	Yes
North	Azkorte	AZK	43.23445	−2.16249	525	N	<i>Q. robur</i>	Yes	Yes	Yes
North	Barrio	BRR	43.06744	−4.66938	1010	NW	<i>Q. pyrenaica</i> <i>Q. petraea</i>	Yes	Yes	Yes
North	Gordoa	GOR	42.92249	−2.37039	780	S	<i>Q. pyrenaica</i> <i>Q. petraea</i>	Yes	Yes	Yes
North	Jugatxi	JUG	42.9488	−2.79952	750	Flat	<i>Q. pyrenaica</i> <i>Q. robur</i>	Yes	Yes	Yes
North	Monte Aa	MAA	43.26972	−4.30201	447	S	<i>Q. robur</i>	Yes	Yes	Yes
North	Muniellos 1	MNL	43.0243	−6.69695	750	E	<i>Q. petraea</i>	Yes	Yes	No
North	Muniellos 2	MUN	43.0316	−6.6835	830	NW	<i>Q. petraea</i>	Yes	Yes	No
North	Muniellos 3	MUS	43.0371	−6.6949	875	NE	<i>Q. petraea</i>	Yes	Yes	No
North	Oiartzun	OIR	43.3108	−1.86754	100	Flat	<i>Q. robur</i>	Yes	Yes	Yes
North	Onquemada	ONQ	43.09215	−4.71636	1116	SE	<i>Q. petraea</i>	Yes	Yes	Yes
North	Orticeo	ORT	43.1691	−4.53718	1170	S	<i>Q. pyrenaica</i>	Yes	Yes	Yes
North	Oteo	OTE	42.71844	−2.39055	960	Flat	<i>Q. faginea</i>	Yes	Yes	Yes
North	Pedroso	PDR	43.21550	−3.86496	370	NE	<i>Q. robur</i>	No	Yes	No
North	Sakana	ALB	42.8955	−2.04957	540	S	<i>Quercus</i> sp.	Yes	Yes	Yes
North	San Vicente de Arana	SVA	42.73285	−2.35063	870	W	<i>Q. faginea</i>	Yes	Yes	Yes
North	Uzkanga	UZK	43.29435	−2.30916	90	Flat	<i>Q. robur</i>	Yes	Yes	Yes
Central System	Arroyofrio	ARF	40.79416	−3.98519	1900	SE	<i>P. sylvestris</i>	Yes	Yes	Yes
Central System	Espinar	ESP	40.7838	−4.1333	1750	W	<i>P. sylvestris</i>	Yes	Yes	Yes
Central System	Fuenfria	FFR	40.78779	−4.04061	1950	N	<i>P. sylvestris</i>	Yes	Yes	Yes
Central System	Hoyos del Collado	HDC	41.00209	−3.89764	1745	W	<i>Q. petraea</i>	No	Yes	Yes
Central System	La Jarosa	JRS	40.66301	−4.15988	1375	E	<i>P. nigra</i> <i>P. sylvestris</i>	Yes	Yes	Yes
Central System	Navafria las Barrigas	NLB	41.00133	−3.83889	1950	W	<i>P. sylvestris</i>	No	Yes	Yes
South	La Cabrilla	CBR	37.84002	−2.83434	1840	E	<i>P. nigra</i>	Yes	No	No
South	Linarejos	LIN	37.92205	−2.90781	1250	NW	<i>P. nigra</i>	Yes	Yes	Yes
South	La Sagra	LSA	37.93473	−2.58724	1760	E	<i>P. nigra</i>	Yes	No	Yes
South	Navanoguera	NAV	37.93215	−2.80704	1640	Valley	<i>P. nigra</i>	Yes	Yes	Yes
South	Poyos de la Mesa	PMB	37.89335	−2.9125	1560	NW	<i>P. nigra</i>	Yes	No	No

16th-century Spanish merchant vessel recorded as having wrecked in the same location) (Watson and Gale, 1990; Dunkley, 2001; Plets et al., 2007). Four other shipwrecks were sampled in order to test whether they could be identified as Iberian-built vessels: Cee I and Cee II, located at the Cee Bay, Spain; the Barreiros, a potential 15th-century clinker-built vessel that appeared after a storm in 2015 at the beach of Barreiros, Spain; and the Belinho, a historic shipwreck washed ashore in Belinho, Portugal (Martins et al., 2020). Finally, one shipwreck not likely to have been built in the Iberian Peninsula was added to the set for analyses: the Ribadeo (16th-century galleon, Ribadeo, Spain), probably identified as the *Santiago de Galicia*, which was built in Naples in the early 1590s (San Claudio Santa Cruz et al., 2013; Eguiluz Miranda et al., 2020). From each shipwreck, 3–30 samples were removed from structural timbers recorded *in situ* (Fig. 1d and e), with the exception of the Belinho timbers, for which the archaeological context is unknown. Sampling protocols and guidelines followed Rich et al. (2018) and Domínguez-Delmás et al. (2019), so that cross-sections of well-preserved structural timbers were prioritized for sampling. Timbers were thoroughly recorded prior to the irreversible sampling procedure, which required the removal cross-sections with a handsaw. Wood identification of deciduous oak (*Quercus* subg. *Quercus*) samples was done visually on the spot, as the large multiseriate rays and the ring-porous disposition of the vessels are key features of this group easily recognisable by the experienced eye. Identification of other species was made through examination of thin sections under a microscope, and wood anatomical features were correlated with those of species detailed in Schweingruber (2001). Deciduous oaks (*Quercus* subg. *Quercus*) and *Pinus sylvestris/nigra* cannot be identified down to the species level based on wood anatomical features

(Schweingruber, 2001); therefore, the archaeological samples retrieved from the shipwreck timbers were identified only as deciduous oak or pine. Three samples of conifer wood could not be identified due to the bad preservation of the wood and the difficulty to obtain optimal thin sections for the observation of anatomical features. They were left unidentified (Table S1 in Supplementary Material). Samples removed from these eight shipwrecks were then subdivided and distributed for tree-ring measurements, and Sr isotope and organic compound analyses, as described in Sections 3–5.

### 3. Dendrochronological approach to timber provenance

One of the pillars of dendrochronology is that trees of the same species growing at the same site will be influenced by the same climatic conditions (mainly temperature and precipitation), and will therefore produce similar growth patterns (Fritts, 1976; Schweingruber, 1996). Those synchronous growth patterns can be cross-matched (i.e. cross-dated) to develop a reference ring-width chronology anchored in the present, which represents the growth of a given species at a specific site. Such reference chronologies are then used to absolutely date wooden artefacts and (pre)historical timbers and, by inference, to establish the provenance of the wood. Growth patterns reflect not only large-scale climatic components (e.g., seasonal cycles, precipitation, temperature trends, volcanic eruptions), but also the climatic and ecological conditions of the immediate environs (e.g., altitude, drought, early or late frosts, flooding, parasite outbreaks, fires, and forest clearance) (Schweingruber, 1996). Therefore, provenance is generally based on the correlation between the artefact's tree-ring patterns and reference

chronologies of known locations (provided these are available). The chronology showing the strongest similarity, expressed as statistical correlation using Student's *t*-value (Baillie, 1982), with the tree-ring series of the sample under study is generally considered to represent the area of origin (for nuances and limitations to the method see Bridge, 2012; Domínguez-Delmás, 2020).

In the Iberian Peninsula, most reference chronologies existing at the beginning of this study had been developed for ecological and/or climatological studies (Gazol et al., 2018). Chronologies developed for ecological studies generally extend back only some 150 years, limiting their applicability to date historical timbers. On the other hand, chronologies developed for climate studies cover several centuries back in time. Yet, these chronologies are typically derived from trees growing at high elevation sites in areas unlikely to have supplied wood for historical construction projects (Domínguez-Delmás et al., 2015). As the dynamics of pine growth vary strongly with altitude in the Iberian Peninsula (Richter et al., 1991; Domínguez-Delmás et al., 2013), these high-elevation chronologies may not serve to date wood from low-elevation sites. There is therefore a strong need for long-span chronologies that represent tree growth from past timber-source areas.

To achieve such a set of reference chronologies from living trees, we followed the sampling strategy outlined above, and selected 23 forest sites. We collected and analysed 1243 samples from 630 trees, measured their rings using a TimeTable device (VIAS, University of Vienna) and built 29 chronologies. We standardised the data applying a 10-year cubic smoothing spline and used a bi-weight robust mean to compute each chronology using the *dplR* package (Bunn, 2008) in R (R Core Team, 2020). In the north of Spain we developed 19 oak chronologies (*Quercus* spp.), eight of which reach back to the first half of the 16th century (Fig. 2). We also developed five black pine (*P. nigra*) chronologies in southern Spain, two of which go back to the 14th century and beyond. Another five pine chronologies, four of Scots pine (*P. sylvestris*) and one of black pine were obtained for the Central System, reaching back to the late 15th and early 16th century (Fig. 2).

Diverse statistics were calculated to describe the quality of the chronologies (Table 2). When considering the portion achieving a subsample signal strength (SSS) higher than 0.85, the temporal coverage of most chronologies was shortened by >100 years due to the scarcity of very old trees (e.g. for oaks in Barrio 1, 2 and 3, Gordoia 1, Jugatxi 1 and 2,

Muniellos 1 and 2, Onquemada and Ortimeo; for pines in La Jarosa 2, La Cabrilla, La Sagra and Navanoguera). The mean correlation between radii within the same tree ( $r_{wt}$ ) is quite high for the oaks (0.588), with the minimum in Barrio 1 ( $r_{wt} = 0.457$ ) and the maximum in Azkorte ( $r_{wt} = 0.786$ ). These values are consistent with what has been reported for *Q. robur* and *Q. petraea* in Eastern Europe (e.g. Nechita et al., 2018), illustrating the range of correlations that could be expected for historic samples derived from the same tree. The correlations between individual oak trees within a site ( $r_{bt}$ ) is much lower at all sites, ranging between 0.151 (Barrio 3) and 0.428 (Azkorte). The low  $r_{bt}$  at Barrio 3 can be explained by the fact that this chronology contains trees that could not be identified with certainty as *Q. pyrenaica*, *Q. petraea* or hybrids (Valbuena-Carabana et al., 2005), and having both species mixed in this chronology could lower the common growth signal. The remaining values for oaks are in range with other studies in North of Spain and Eastern Europe (e.g. Rozas et al., 2009; Netsvetov et al., 2017). These rather low values imply a low common growth signal, which could hamper dating or provenancing individual archaeological/historical samples and hinder the development of floating object chronologies, even if timbers in a given structure or building phase originate from the same forest.

Pines, in general, show a high mean intra-tree correlation ( $r_{wt} = 0.616$ ) and a coherent inter-tree correlation ( $r_{bt}$ ), which ranges between 0.289 (PISY, Fuenfria) and 0.441 (PINI, Navanoguera). High crossdating values can therefore be expected for archaeological and historical samples derived from the same tree, and relative/absolute dating of timbers with trees/chronologies from the same site should not be too challenging in most cases (see e.g. Domínguez-Delmás et al., 2018).

In a next step, we explored tele- and heteroconnections between the different standard chronologies to assess the potential of developing regional and/or composite (multi-species) chronologies. For this, hierarchical clusters (HC) and correlation coefficients were calculated for the two groups of chronologies (oaks and pines), considering their respective common intervals (1924–2014 for the oaks and 1798–2014 for the pines). For the hierarchical cluster we used  $1/t$  as a distance measure and grouped the chronologies according to the unweighted pair group method with arithmetic mean (UPGMA) (García-González, 2008). Although such a clustering analysis does not provide a measure of the significance of the results, adding *a priori* discriminating factors such as gradients (longitudinal, latitudinal, altitudinal) or different species

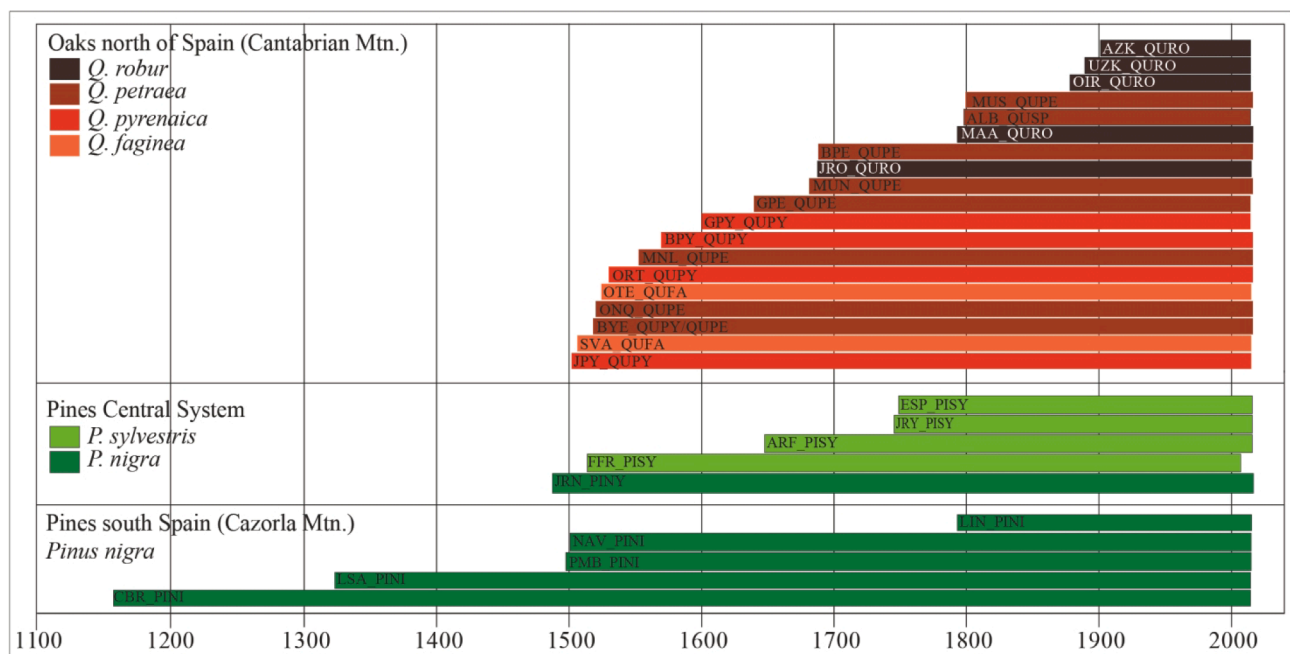


Fig. 2. Total length covered by the tree-ring chronologies developed from living trees of different species in the three targeted areas.

**Table 2**

Descriptive information of the standard tree-ring chronologies. Standard chronologies were computed in dplR (Bunn, 2008) applying a 10-year smoothing cubic spline. Date begin: first year of the total length;  $r_{wt}$ : correlation of cores within trees;  $r_{bt}$ : correlation between trees; SSS: period in which the subsample signal strength has a value higher than 0.85 (Buras, 2017).

Region	Site	Chrono code	Species	N trees	N radii	Date begin	Date end	$r_{wt}$	$r_{bt}$	SSS > 0.85
North	Azkorte	AZK	<i>Q. robur</i>	7	16	1901	2014	0.786	0.428	1926–2014
North	Barrio 1	BPE	<i>Q. petraea</i>	10	21	1688	2016	0.457	0.235	1813–2016
North	Barrio 2	BPY	<i>Q. pyrenaica</i>	6	13	1569	2016	0.511	0.249	1720–2016
North	Barrio 3	BYE	<i>Q. pyrenaica/petraea</i>	6	21	1518	2016	0.624	0.151	1743–2016
North	Gordoa 1	GPE	<i>Q. petraea</i>	6	17	1644	2015	0.566	0.26	1883–2015
North	Gordoa 2	GPY	<i>Q. pyrenaica</i>	17	43	1600	2015	0.601	0.289	1674–2015
North	Jugatxi 1	JPY	<i>Q. pyrenaica</i>	10	21	1501	2015	0.525	0.268	1707–2015
North	Jugatxi 2	JRO	<i>Q. robur</i>	10	19	1687	2015	0.627	0.241	1833–2015
North	Monte Aa	MAA	<i>Q. robur</i>	16	31	1793	2016	0.695	0.383	1855–2016
North	Muniellos 1	MNL	<i>Q. petraea</i>	9	21	1552	2016	0.585	0.245	1814–2016
North	Muniellos 2	MUN	<i>Q. petraea</i>	20	43	1681	2016	0.608	0.363	1793–2016
North	Muniellos 3	MUS	<i>Q. petraea</i>	21	43	1799	2016	0.576	0.414	1844–2016
North	Oiartzun	OIR	<i>Q. robur</i>	10	19	1878	2014	0.563	0.242	1888–2014
North	Onquemada	ONQ	<i>Q. petraea</i>	16	32	1520	2016	0.503	0.294	1631–2016
North	Orticeo	ORT	<i>Q. pyrenaica</i>	18	43	1530	2016	0.61	0.31	1641–2016
North	Oteo	OTE	<i>Q. faginea</i>	25	60	1524	2015	0.53	0.291	1589–2015
North	Sakana	ALB	<i>Quercus</i> sp.	21	50	1798	2014	0.579	0.257	1832–2014
North	San Vicente de Arana	SVA	<i>Q. faginea</i>	24	58	1506	2015	0.515	0.304	1553–2015
North	Uzkanga	UZK	<i>Q. robur</i>	9	18	1889	2014	0.707	0.305	1910–2014
Central System	Arroyofrío	ARF	<i>P. sylvestris</i>	83	116	1648	2015	0.556	0.322	1707–2015
Central System	Espinar	ESP	<i>P. sylvestris</i>	15	25	1748	2015	0.615	0.311	1767–2015
Central System	Fuenfría	FFR	<i>P. sylvestris</i>	31	54	1515	2015	0.495	0.289	1584–2015
Central System	La Jarosa 1	JRN	<i>P. nigra</i>	27	54	1488	2015	0.627	0.408	1517–2015
Central System	La Jarosa 2	JRY	<i>P. sylvestris</i>	14	28	1745	2015	0.592	0.363	1847–2015
South	La Cabrilla	CBR	<i>P. nigra</i>	35	78	1165	2014	0.595	0.308	1531–2014
South	Linarejos	LIN	<i>P. nigra</i>	64	90	1795	2014	0.685	0.423	1821–2014
South	La Sagra	LSA	<i>P. nigra</i>	20	53	1324	2014	0.65	0.396	1584–2014
South	Navanoguera	NAV	<i>P. nigra</i>	63	122	1501	2014	0.669	0.422	1678–2014
South	Poyos de la Mesa	PMB	<i>P. nigra</i>	17	34	1498	2014	0.668	0.409	1508–2014

facilitates the interpretation of results.

The oak chronologies clearly separate into two main groups, namely the coastal and inland sites (Fig. 3). The coastal sites are represented by three chronologies of *Q. robur* (Fig. 3a and b), which show low inter-site correlations (Fig. 3c). This result suggests that dating historic timbers of *Q. robur* grown at such coastal sites will be challenging. The group comprising inland sites is divided into two subgroups, one (2a) that includes *Q. petraea* and *Q. pyrenaica* chronologies located in the centre and the west of the Cantabrian Mountains, and another subgroup (2b) that includes all inland chronologies in the eastern part of the Cantabrian Mountains, regardless of the species (Fig. 3a and b). Within subgroup 2a, the western sites (MUN, MUS, MNL) could be considered one *Q. petraea* chronology given the high correlations between the three sites and their close proximity. A composite *Q. petraea* – *Q. pyrenaica* chronology could be made with the rest of the inland sites of subgroup 2a (BPE, ONQ, ORT, BPY, BYE). Similarly, the high correlations within group 2b (>0.7) between ALB, GPY, OTE and SVA (Fig. 3c) also indicate the potential to build a composite regional chronology of *Q. petraea*, *Q. pyrenaica* and *Q. faginea*, which could be tentatively expanded to include the rest of the sites in group 2b. Consequently, dating of historic timbers should be feasible when the wood originated from inland sites and the chronologies (also the object-chronologies) have an optimal sample depth (Table 2), but the differentiation of species based on crossdating scores seems *a priori* very challenging and should be further explored.

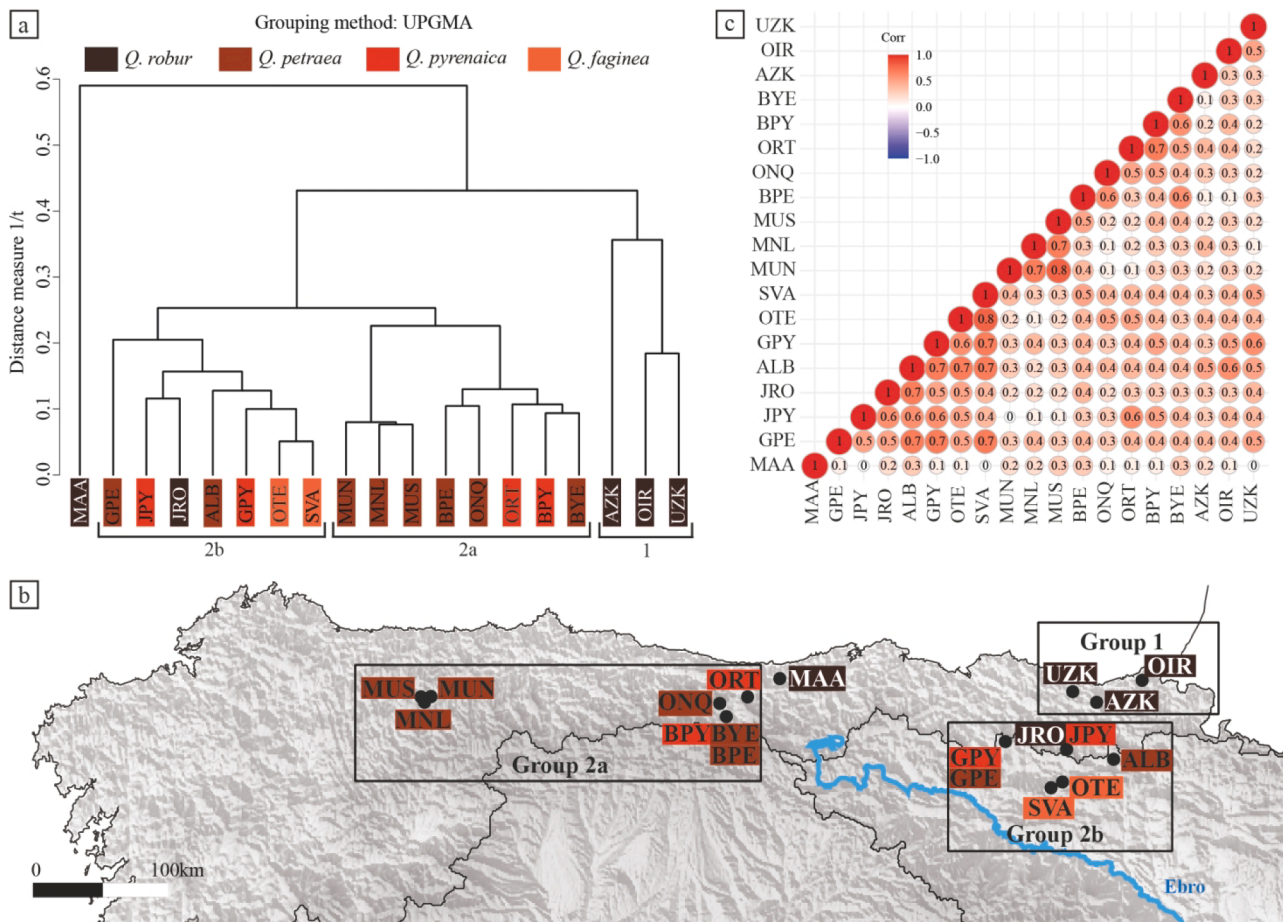
Interestingly, the MAA chronology (*Q. robur*) was not assigned to any group. This outlying position and its low correlations with other chronologies indicate that this chronology has a low potential to be used as a reference chronology for dating purposes (Fig. 3). MAA was the only site still managed for timber production, which could explain the lack of correlations with the other sites.

The pine chronologies also separate into two well defined groups, one represented by the *P. sylvestris* chronologies developed in the northern face the Central System (group 1, Fig. 4a and b), and another one grouping the rest of the chronologies to the south of the Central

System. Interestingly enough, the *P. sylvestris* and *P. nigra* chronologies from the JRS site in the centre of Spain have both a stronger common signal with the *P. nigra* chronologies from the south of Spain than with the nearby *P. sylvestris* chronologies from the Central System. This suggests that climate conditions are very different in the north/south faces of the Central System, the southern ones being more similar to those of the Cazorla Mountains (south of Spain), which results in the trees showing a similar response than the pines in the south, regardless of the species. For dendroarchaeological studies, the low correlations ( $r < 0.3$ ) between the *P. sylvestris* chronology at JRY and the other three *P. sylvestris* chronologies indicates that it will not be possible to date historic timbers of the same species from one side of the Central System with chronologies from the other side. Species identification based on dating scores will only be attained when the reference chronologies represent defined mono-specific sites (Domínguez-Delmás et al., 2017, 2018; Sánchez-Salguero et al., 2017).

Once the reference chronologies were completed, we compared the tree-ring series obtained from shipwreck samples with the newly developed chronologies of the corresponding genus. This comparison, known as crossdating (Douglass, 1941; Baillie, 1982; Pilcher, 1990), resulted in the dating of three oak timbers from the Magdalena shipwreck with a *Q. petraea* chronology that combined trees from Barrio 1, Gordoa 1, Muniellos 1 and 2 and Onquemada in the centre and west of northern Spain (Table S2 in Supplementary Material; for details see Trindade et al., 2020). The tree-ring series obtained from those timbers (two frames and an outer hull element) had been internally crossdated (crossmatched between them) and merged into an object-chronology, MAG3MC, that spanned 127 years. This object chronology was dated to the year 1716 CE. The rest of the shipwreck samples remain undated, with only two oak timbers from Barreiros (M07 and M11) and two others from Belinho (BEL01-013W-01S and BEL01-024W-01S) crossmatching relatively between them (not shown). The lack of more crossmatches between samples from the same shipwreck could be due to the different geographical origin of the wood, or timber derived from forests with different management regimes (as illustrated by the clustering position





**Fig. 3.** Dendrogram (a) and correlation coefficients (b) calculated for the common interval of the oak standard chronologies (1924–2014); c) geographical location of the groups defined by the hierarchical cluster. The dendrogram shows the results of a hierarchical cluster analysis considering  $1/t$  as distance measure and unweighted pair group method with arithmetic mean (UPGMA) as described by García-González (2008).

of the MAA chronology), or caused by the high variability between trees growing in one site (illustrated by the low correlations between some of our oak sites). The possibility that some of the sampled timbers were not contemporary (i.e. were not part of the original construction) cannot be discarded either. Reasons for the general low dating success include the possibility that i) the tree-ring series of the ship timbers do not reflect the growth pattern common to the trees included in the chronologies (e.g., due to different forest use), ii) the chronologies developed do not reflect the exact source areas (e.g. inland chronologies versus coastal source areas), or iii) the shipwreck oak samples analysed were not originated from Iberian parent trees. Also important is the fact that, from the 84 shipwreck oak samples collected, only 59 were included in the dendrochronological study, with the rest having between 4 and 40 rings. The objectives of the ForSEAdiscovery project comprised the characterization of trees used during the Early Modern Period for shipbuilding, therefore samples with less than 40 rings were sometimes also collected, and they were measured to acquire growth rates of the trees used for specific timber elements, or when there was a chance to compare them with longer tree-ring series of samples from the same wreck. In our oak dataset, from the 59 samples analysed, seven had less than 40 rings, 27 had between 40 and 80 rings, and only 15 samples (25%) had more than 100 rings (Table S1 in Supplementary Material), which contributes to the low ratio of dated samples.

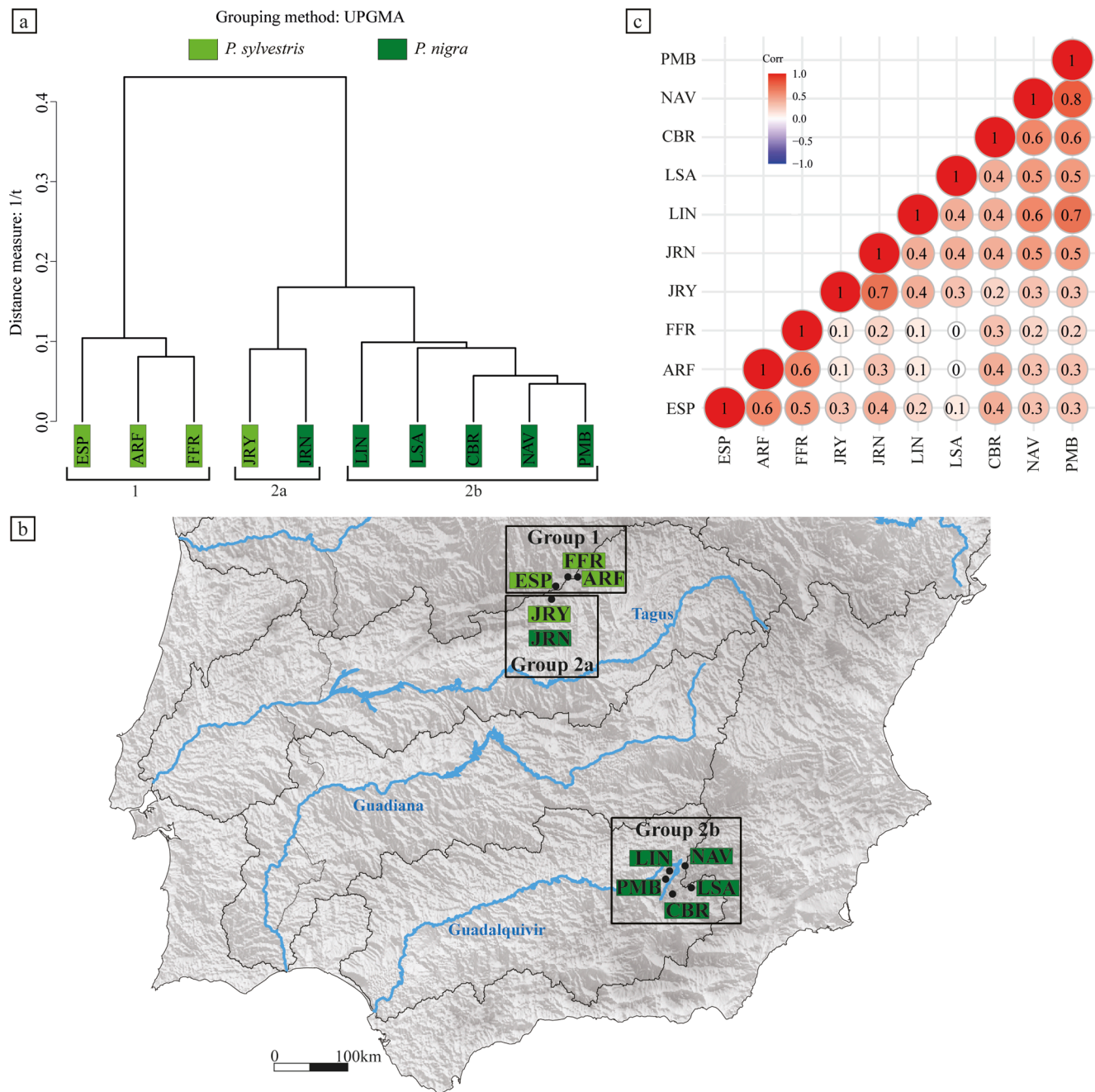
#### 4. Strontium isotopes as wood provenance markers

Different approaches can be used to study wood provenance, but most of them are based on tracers controlled by climatic factors

(Dormontt et al., 2015). The analysis of strontium (Sr) isotopes in wood offers an interesting opportunity to discriminate between different wood sources according to geological parameters (Hajj et al., 2017). Indeed, Sr is one of the most abundant trace elements, ubiquitous in rocks and released into waters and soils by weathering processes. As Sr is an analogue of calcium (Ca), it is taken up from soils by plants to be used in cell wall construction, and subsequently ends up incorporated into animals through the food chain (Burger and Lichtscheidt, 2019).

Strontium has four naturally occurring stable isotopes, with approximate abundances of 0.56% ( $^{84}\text{Sr}$ ), 9.87% ( $^{86}\text{Sr}$ ), 7.04% ( $^{87}\text{Sr}$ ) and 82.53% ( $^{88}\text{Sr}$ ). The radioactive decay of rubidium-87 ( $^{87}\text{Rb}$ ; half-life of  $4.9 \times 10^{10}$  years) produces the radiogenic isotope  $^{87}\text{Sr}$  that is continuously being added to the initial amount of  $^{87}\text{Sr}$  in a given rock. The more the rock is aged and initially rich in Rb, the higher is its  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio (Faure, 1986; Capo et al., 1998). As physicochemical and biological processes (especially weathering and uptake) do not affect the measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio, the Sr isotopic signature in plants should be (at least partially) derived from this specific  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the rocks underlying them (Burger and Lichtscheidt, 2019). This chemical correspondence between plants and the geological features upon which they grow is why Sr isotope ratios have the potential to be used as markers of wood provenance (Hajj et al., 2017).

Despite being used in diverse archaeological studies,  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios have seldom been used to trace the provenance of archaeological wood (Reynolds et al., 2005), and especially wood from shipwrecks (Rich et al., 2016). Recent advances in mass spectrometry now allow the detection of Sr isotopes' mass-dependent fractionation, measured as  $\delta^{88}\text{Sr}/^{86}\text{Sr}$ . Some recent studies demonstrate significant variations of  $\delta^{88}\text{Sr}/^{86}\text{Sr}$



**Fig. 4.** Correlation coefficients (a) and dendrogram (b) calculated for the common interval of the pine standard chronologies (1798–2014); c) geographical location of the groups defined by the hierarchical cluster. The dendrogram shows the results of a hierarchical cluster analysis considering  $1/t$  as distance measure and unweighted pair group method with arithmetic mean (UPGMA) as described by García-González (2008).

values according to the trophic level (Knudson et al., 2010), and between plant and soil compartments (de Souza et al., 2010; Bullen et al., 1997). Then  $\delta^{88/86}\text{Sr}$  values could be used in conjunction with the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratio to improve constraints on the sources of Sr in the archaeological materials being studied.

The relevant hypothesis under scrutiny within our study from an isotopic point of view was that trees growing on specific rock and soil formation types in the Iberian Peninsula have specific Sr isotopic signatures and can be an indicator of timber provenance (Hajj, 2017). Two tree genera (*Pinus* and *Quercus*) were targeted from 26 Spanish forest stands that were considered potential sources of wood between the 16th and 18th centuries, using the site and sampling selection procedures described in point 2. These trees grew on relatively thin soils, originating from different rock types (siliciclastic, carbonate, and metamorphic

rocks) (Table 3; Figs. S1 and S2 in Supplementary Material). At these sites,  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{88/86}\text{Sr}$  values of bulk and “available” pool from soils and rocks were measured, in addition to those in the wood sampled from living trees growing on these soils. The rock types and ages were characterized and the link between the  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{88/86}\text{Sr}$  in rocks, soils, and trees studied for each sampling site, which in turn produced a local Sr isotopic signature necessary to determine the provenance of archaeological wood.

Our results indicate that  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratios in oak and pine trees reflect the signature of the corresponding soil exchangeable pool. This is not the case with  $\delta^{88/86}\text{Sr}$  signatures. Differences between soils and oak trees  $\delta^{88/86}\text{Sr}$  values indicate mass-dependent fractionation, with trees taking up lighter ( $^{86}\text{Sr}$ ) isotopes and leaving the soil exchangeable pool enriched with the heavier isotopes ( $^{88}\text{Sr}$ ). This fractionation observed

**Table 3**

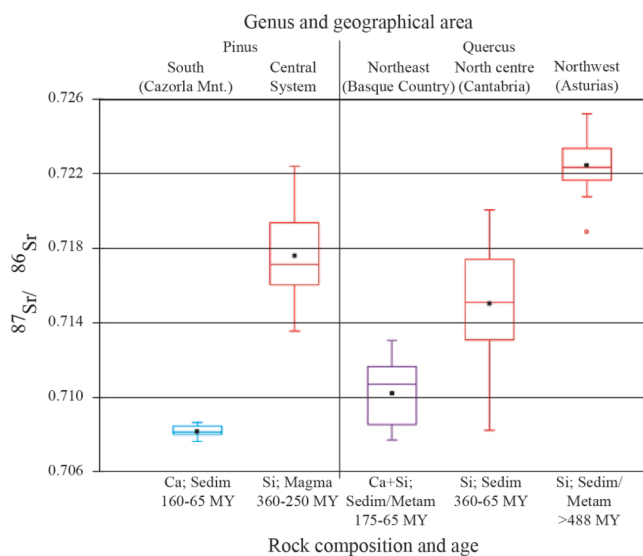
Rock types and ages of the 26 studied stands. Facies of sedimentary rocks are given according to classification (i) of marine carbonate rocks (Dunham, 1962) and (ii) of granulometric classification (Wentworth, 1922) for detrital rocks. Data in italic were found from geological map information. All other rocks are described from samples collected on the field.

Region	Rock types		Facies	Age	Site
Northwest (Asturias)	Sedimentary	Silicate/Mica schist/	Mudstone and siltstone	Cambrian	MUS
	/Metamorphic	Quartzite			
	Sedimentary	Silicate	Sandstone (with muscovite and chlorite)	Cambrian	MUN
North centre (Cantabria)	Metamorphic	Quartzite	Some biotites	Cambrian	MNL
	Sedimentary	Silicate	Siltstone/Sandstone	Middle Jurassic	PDR
			Fine sandstone	Trias	MAA
			Sandstone and conglomerate	Carboniferous	ONQ
			Siltstone and sandstone locally metamorphised	Carboniferous	BRR
			Mudstone and siltstone/Sandstone	Carboniferous	ORT
			Mudstone and siltstone	Upper Cretaceous	AST
			Mudstone	Upper Cretaceous	UZK
				From lower Jurassic to middle Jurassic	AZK
Northeast (Basque Country)			Mudstone/Packstone/Grainstone	Upper Cretaceous	SVAOTE
		Carbonate/Silicate	Mudstone/Siltite	Upper Cretaceous	JUG
		Silicate	Mudstone/Siltstone/Standstone	Upper Cretaceous	OIR GOR
		Schist/Mica schist	–	Variscan	ART
	Metamorphic	<i>n.d.</i>	–	Upper Cretaceous	ALB
	Sedimentary	<i>Orthogneiss</i>	–	> Variscan	HDC
	Magmatic	Granite	Rich in biotite	Variscan	NLB
		Granite/diorite	Locally amphibole rock	Variscan	FFR
		Granite	Rock locally rich in feldspars and chlorite	Variscan	ARF
		Granite	Locally amphibole rock	Variscan	JRS
		Magmatic/Metamorphic	Granite/gneiss	> Variscan	ESP
Andalusia	Sedimentary	Carbonate: limestone	Mudstone/Wackestone/Grainstone	Late Jurassic to lower Cretaceous	LIN
		Carbonate: limestone ± dolostone	Mudstone/Grainstone	Upper Cretaceous	NAV

for oak trees was not found in pines, suggesting that the isotopic fractionation during tree uptake is species dependent (Hajj, 2017). These results further confirm the possibility of significantly differentiating the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic signature of xylem from the three different Spanish areas tested (north, centre and south of Spain) (Fig. 5), and even from the three regions within the northern area (Asturias in the northwest, Cantabria in the north-centre, and Basque Country in the northeast) (Fig. 6), where  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios show significant differences between groups of stands, regardless of the species. This demonstrates high

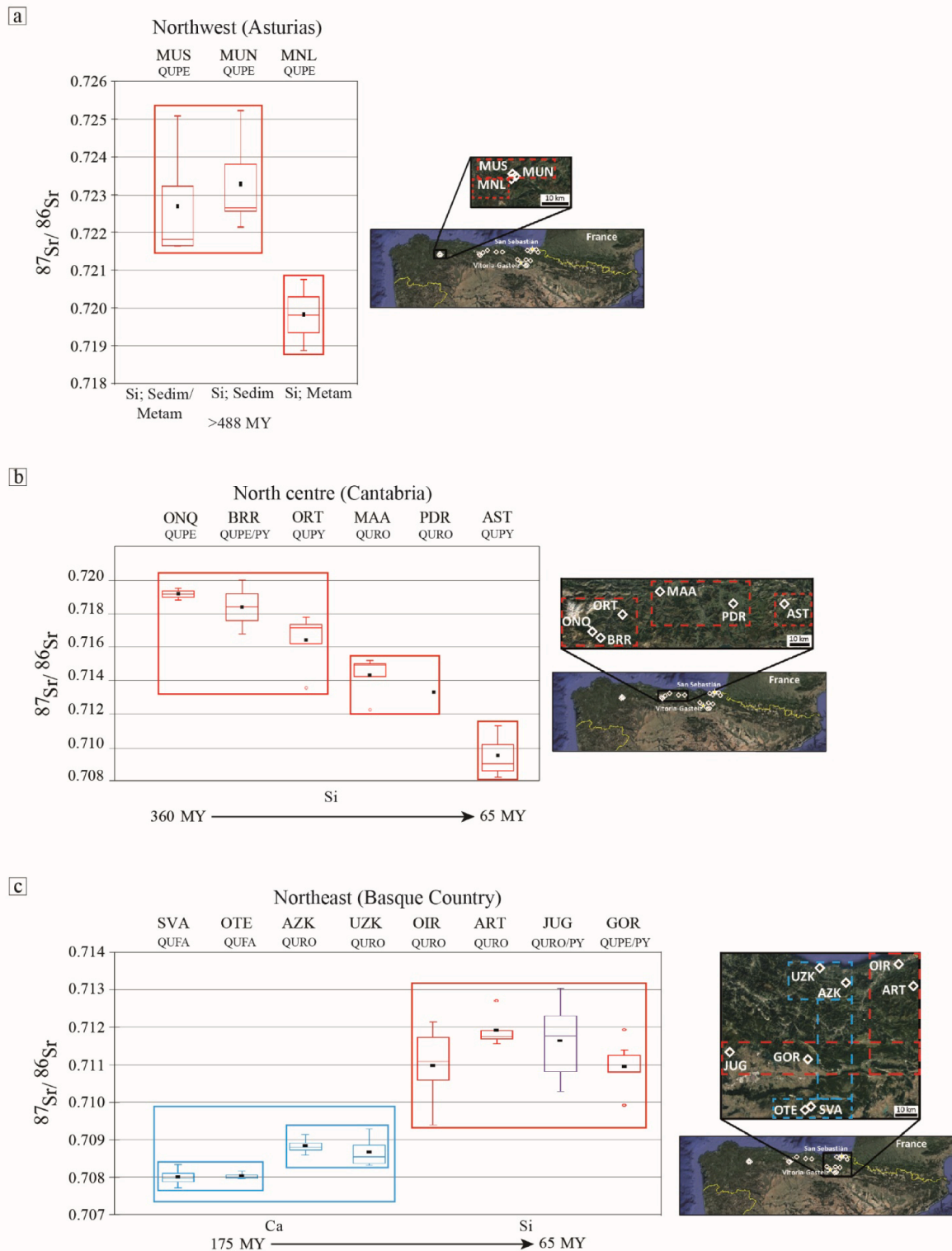
potential of using  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic signatures in trees to discriminate local geographic areas for provenancing. Pine  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in the Central System also show differentiated signals, but present more complexity and should be further explored (Fig. 7). The stands in the south (Cazorla Mountains) do not present significantly different signals, although the high differences with trees in the Central System allows differentiating wood from those two areas. The  $\delta^{88/86}\text{Sr}$  measured in trees showing species-dependent isotopic fractionation can be used to explore intra-site signatures. For example, the  $\delta^{88/86}\text{Sr}$  values allow for the discrimination of wood from different tree species growing in the same type of soil, even if their  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio is similar (Fig. 8).

Our results suggest that the chemical and isotopic composition measured in the archaeological woods from the shipwrecks we studied were all contaminated during the centuries of submersion in seawater (Hajj et al., 2017). Marine Sr was found to be adsorbed on the wood or included in the minerals precipitated during waterlogging processes, thereby changing the original Sr isotopic signature. Several extraction experiments were tested, and an adapted protocol was developed to extract seawater elements and to retrieve the original signature of the archaeological wood (see Appendix A in Hajj et al., 2017). We succeeded to validate an extraction protocol to retrieve the original signature of one wood sample. However, our measurements of  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{88/86}\text{Sr}$  values in different points of an oak shipwreck timber also indicated that most of the timber did not conserve the original Sr. Prior to apply this decontamination protocol, it is required to check if the values close to the surface were more similar to those of seawater than the values of the inner part of the timber (Hajj et al., 2017, p. 40). These results imply that the approach is not widely applicable, being more or less in agreement with those found by Rich et al. (2016) on shipwrecks on the Eastern Mediterranean that had also been submersed in seawater for centuries. To determine the provenance of wood sampled from shipwrecks using Sr isotopes, it is crucial to identify the degree and type of contamination of waterlogged woods and to examine whether the Sr initially taken up by the trees is conserved (the “memory of the wood”) and in which wood



**Fig. 5.** Pine and oak wood  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios according to contrasted rocks composition and ages. The values show discrimination at regional scale. Ca, carbonate rocks; Si, silicate rocks; Sedim, Sedimentary; Metam, metamorphic; MY, age of the rocks in million years.



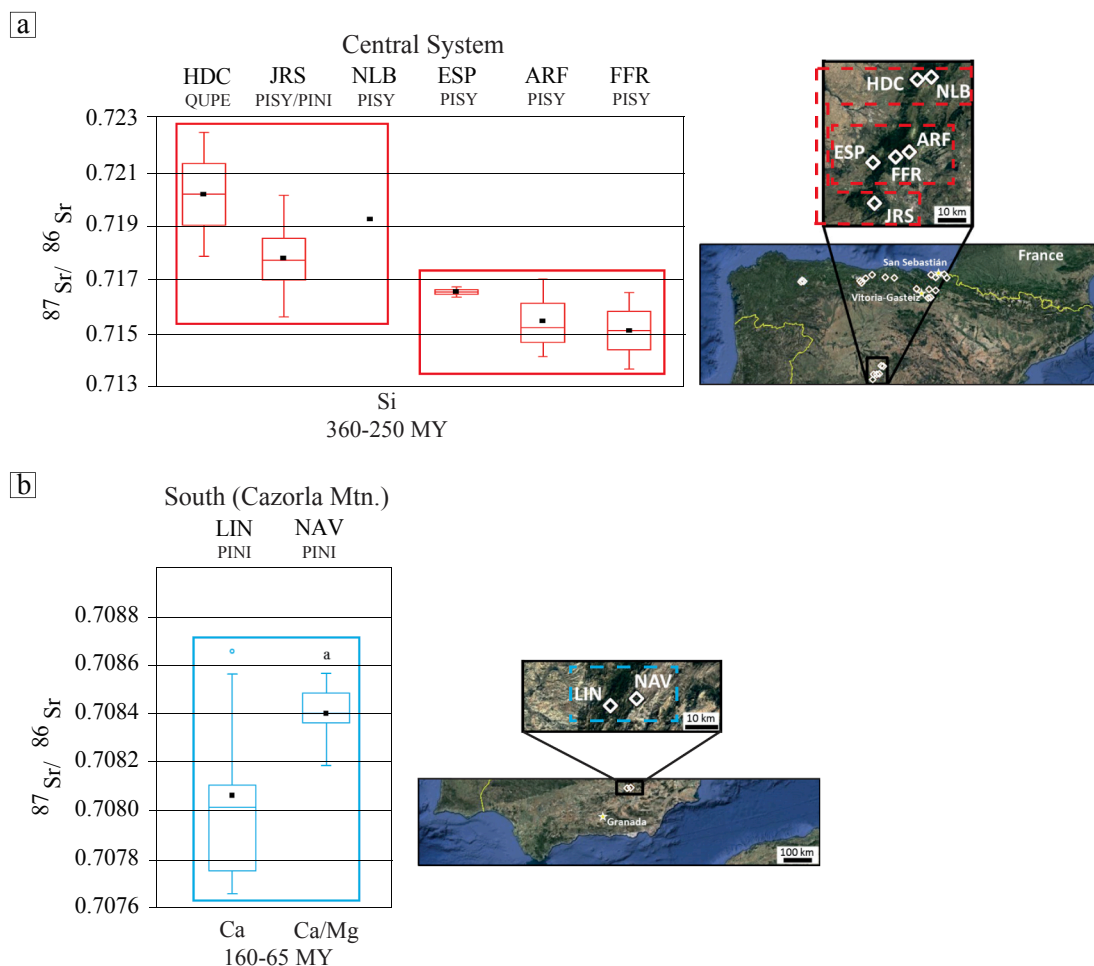


**Fig. 6.**  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of oak samples indicating discrimination at stand level. a) variation of ratios from wood samples in sites from the northwest (Asturias) growing on silicate rocks of very old age, where two subgroups can be identified; b) the trend of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from the six stands on silicate rocks of different ages in the north centre (Cantabria) shows significant differences between tree signatures according to stands, illustrating the high potential of using  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic signatures in trees to discriminate local geographic areas for provenancing; c)  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of wood from trees in the northwest (Basque Country), showing a good discrimination between two groups growing in carbonate rocks, and no significant discrimination between the wood from sites on silicate rocks.

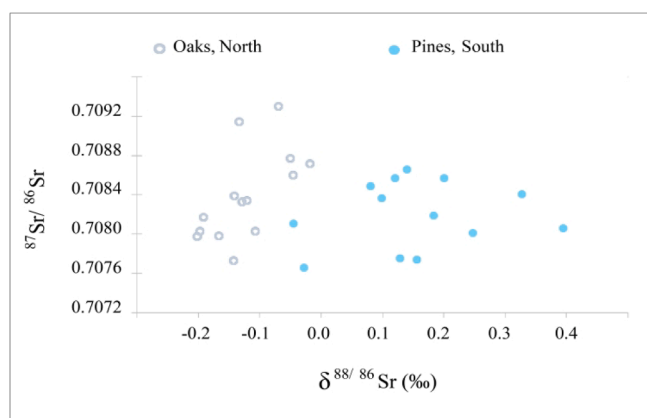
tissues it is retained. Studying shipwreck wood samples of known provenance would be the best approach to develop and validate protocols designed to eliminate contamination and restore the original signature of the wood.

## 5. Organic chemical composition of wood: an approach for ship timber provenance studies

Wood is a complex composite made up of polymers of cellulose,



**Fig. 7.**  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of pine samples at stand level. a) Wood from stands in the Central System located on silicate rocks with the same mineralogical composition and age separates into two groups (notice that stand HDC is composed of *Q. petraea*, representing an exception in the Central System; the Sr isotopic signature is species-independent); b) no significant differences have been found between pine  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from the stands in the south (wood from LIN shows strong heterogeneity).



**Fig. 8.** Variation of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios and  $\delta^{88/86}\text{Sr}$  (‰) values measured in trees sampled from one oak stand in the north and one pine stand in the south, both developed on carbonated rocks. When rocks are similar, it is difficult to find a specific stand signature using  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic signatures, but the  $\delta^{88/86}\text{Sr}$  values can then help to discriminate the stands. In the example of this figure, this delta varies because of a species effect (pine and oak trees have different  $\delta^{88/86}\text{Sr}$  values), oak trees taking up lighter ( $^{86}\text{Sr}$ ) isotopes from soil).

hemicellulose, lignin, and extraneous (organic and inorganic) compounds. Further complexity of this lignocellulosic material arises from the different tissues that wood is made of (parenchyma, vessels, fibres, etc.). These tissues have multiple functions in woody plants, such as water and nutrient transport, biochemical storage, and mechanical support (Fengel and Wegener, 1984). Tree taxonomy, as well as environmental conditions at temporal and geographic scales play an important role in the structural and chemical composition of wood (Kranitz et al., 2016). Wood structure and chemical characteristics differ between the two main groups of trees: gymnosperms, which include conifers such as pines, and angiosperms, which include flowering plants such as oaks. Moreover, the chemical composition of wood in a timber is subject to changes caused by external factors that may alter it over time. For example, in the case of underwater archaeological timber, carbohydrate polymers (especially hemicelluloses) are altered, whereas modifications of lignin appear to be less intense (Blanchette et al., 1989). Therefore, identifying and quantifying organic compounds in wood could aid in discriminating between tree species and, consequently, contribute to establish the provenance of archaeological wood.

Fourier transform infrared (FTIR) spectroscopy and Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) are two techniques that have been widely used for wood chemical studies (e.g. Evans, 1991; Moore and Owen, 2001; Chen et al., 2010; Xu et al., 2013 and references therein). These analytical methods require very small quantities of sample and provide detailed information about wood molecular

structure and composition. Additionally, they also permit the assessment of chemical changes in wood induced by environmental conditions during storage, which is especially important when working with archaeological samples (e.g. Wilson et al., 1993; Lucejko et al., 2015). In previous studies, FTIR and Py-GC-MS have been used successfully to discriminate between wood species through contrasting chemical differences, such as the relative proportions of carbohydrates (e.g. polysaccharides) and lignin compounds (Blanchette et al., 1989; Colom et al., 2003; Gandolfo et al., 2016; Popescu et al., 2007). These methods have also been used to gain insights into the pathways occurring during wood degradation processes. As for waterlogged shipwreck wood, polysaccharides appear to be the most vulnerable wood chemical compounds. Several studies have revealed a higher proportion of lignin compounds in these type of samples as a result of the preferential degradation of polysaccharides in such underwater environments (Colombini et al., 2009; Wilson et al., 1993). In fact, understanding wood degradation pathways is relevant to correctly interpret results, especially in studies related to the identification and provenance of archaeological wood. FTIR and Py-GC-MS have also been used to discriminate wood samples based on their geographical origin (Carballo-Meilan et al., 2016; Colom and Carrillo, 2005; Rana et al., 2008; Santoni et al., 2015). This has been possible through the implementation of multivariate analysis (Principal Component Analysis, Discriminant Analysis) to the FTIR and Py-GC-MS data.

In the Iberian Peninsula, the identification of archaeological wood down to the species level acquires paramount relevance when researching shipwreck timbers. The identification of several timbers as an endemic oak species, for example *Quercus faginea* and *Q. pyrenaica*, would indicate that the ship was built in an Iberian shipyard as these species almost exclusively occur in the Iberian Peninsula (Domínguez-Delmás, 2020). To make these species determinations, FTIR was first tested on samples from living trees of several species of pine (*Pinus sylvestris*, *P. nigra*) and oak (*Q. robur*, *Q. petraea*, *Q. faginea* and *Q. pyrenaica*) identified beforehand by the leaves and growing at different sites, in order to understand their chemical composition,

identify differences, and develop references that would allow the subsequent differentiation of shipwreck timbers (Traoré, 2018). Discriminant analyses applied to the FTIR-ATR fingerprints of living pines showed that a discriminant model using lignin bands only separated the samples by location, whereas a model using only polysaccharide bands managed to separate samples by both species and site (Traoré et al., 2018a). Multivariate analyses applied to the FTIR and Py-GC-MS spectra obtained from heartwood samples of the living oaks resulted in the separation of the four oak species under study, and discrimination was based on the two main groups of wood compounds (polysaccharides and lignin) (Traoré et al., 2018b). Furthermore, the characterisation of wood from archaeological samples confirmed the potential of FTIR and Py-GC-MS techniques to assess the degradation of compounds (mainly polysaccharides), and allowed the detection of spectra suitable for identification and provenance studies (Traoré et al., 2016, 2017). Consequently, when these methods were applied to oak wood samples from four different shipwrecks with suspected Iberian origins (Magdalena, Belinho, Ribadeo, and Yarmouth Roads, point 2) it was possible to identify the degradation level of their polysaccharide compounds (Traoré et al., 2018b). However, we were unable to assign an oak species to the shipwreck samples with certainty using discriminant analyses (Fig. 9). For example, based on the FTIR results, none of the samples would be identified as *Q. pyrenaica*, whereas Py-GC-MS detected three samples (RIB08 from the Ribadeo galleon, and YAR07 and YAR19 from the Yarmouth Roads wreck) as likely being from this species (FTIR actually classifies the sample YAR07 very likely as *Q. petraea*). Similarly, the Py-GC-MS technique did not identify any sample as *Q. faginea*, whereas FTIR placed two samples (BEL54 and BEL69 from the Belinho wreck) as likely being from this species (Fig. 9). Still, most of the samples were identified by both methods as being either *Q. robur* or *Q. petraea*, although the two methods disagree in half of the cases on the species assigned to the samples. For two samples (MAG10 and MAG21 from the Magdalena), both methods agree in their classification as *Q. petraea*, which is consistent with the dating of two other oak samples from this shipwreck with chronologies of that species. In fact, the tree-ring

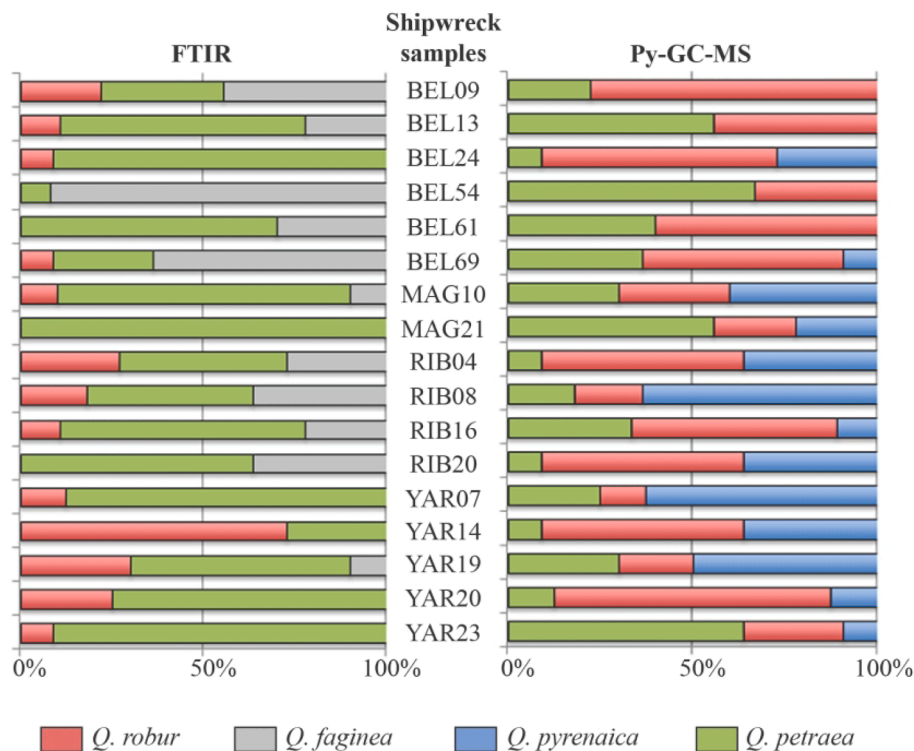


Fig. 9. Species identification (expressed in proportions) of shipwreck samples according to discriminant analyses of FTIR (a) and Py-GC-MS (b) results (adapted from Traoré et al., 2018b, with permission from Elsevier).



chronologies dating those samples derive from Muniellos forest, one of the sites in the north of Spain where *Q. petraea* is the dominant species, and where part of the timber for the construction of this ship was extracted according to historical documents (Trindade et al., 2020).

All in all, these results call for caution when using FTIR and Py-GC-MS to assign species to shipwreck timbers, and highlight the need to continue developing references and carrying out tests on numerous samples to enhance our understanding of the signals still present in shipwreck timbers under different degrees of preservation (Colombini et al., 2007, Traoré et al., 2017).

## 6. Other approaches to timber provenance tested in the Iberian Peninsula

In our project, we also tested the potential to improve dendroprovenancing precision by using chronologies built from variables obtained through quantitative wood anatomy, which in this case involved the variation in earlywood vessel size and latewood width of oaks in nine of our study sites in the north of Spain (Akhmetzyanov et al., 2019), and latewood density of pines (35P. *sylvestris* and 47P. *nigra*) in six of our sites in Central and Southern Spain (Akhmetzyanov et al., 2020). In the north of Spain, anatomical features were measured in 89 oaks of the four species mentioned in point 2 (18 *Q. robur*, 26 *Q. petraea*, 13 *Q. faginea* and 26 *Q. pyrenaica*). Together with new statistical approaches (Principal Component Gradient Analyses, Buras et al. (2016), and Wilcoxon rank sum test), we were able to identify the main climatic factors driving the inter-annual variation of latewood widths and earlywood vessels size. Whereas the former show a strong correlation with average summer (June-July) temperatures in an east-west gradient, the latter does with winter and spring temperatures in a north-south gradient (negative correlation of coastal oaks with February temperatures, and high positive correlation of inland oaks with average March-May temperature) (Akhmetzyanov et al., 2019). Combining these wood-anatomical variables (mainly hydraulically-weighted diameter, a proxy for water conductivity) with ring-width chronologies showed higher provenance accuracy tested with living trees than when using ring-width data only (Akhmetzyanov et al., 2019). The use of year-to-year variation in vessel sizes allowed us to assign locations of individual trees within one sub-region in the northeast (Northern, Central and Southern Basque country, corresponding to sites located near the coast, middle and inland respectively), while the use of latewood width improved the separation of trees between two sub-regions in the north (Cantabria to the west and the Basque country to the east). This improved accuracy arises from the fact that earlywood vessel size and latewood width store climatic signals not recorded in tree-ring widths (Souto-Herrero et al., 2018a). These additional climatic variables (winter and spring temperature for vessel sizes, and summer temperature for latewood width) improve the power to discriminate locations with differing climates from one another. An additional advantage of using quantitative wood anatomy is that certain variables (e.g., total ring width and latewood width) are influenced by forest dynamics and or management, whereas earlywood vessel size is not (Souto-Herrero et al., 2018b). Considering that many of the Iberian forests that still retain old trees underwent intensive anthropogenic alterations, and that wood used for shipbuilding may have come from managed forests, using proxies that are independent of these exogenous forest disturbances is thus highly relevant for provenancing shipwreck timbers. However, the development of such earlywood vessel chronologies is cumbersome, and only a proven success when applied to shipwreck timbers or other type of historical timbers would justify its systematic implementation.

Similarly, the use of latewood density combined with ring-width chronologies in a two-step approach has the potential to improve accuracy of pinpointing the origin of pines in the center and south of the Iberian Peninsula (Akhmetzyanov et al., 2020). Latewood density is strongly influenced by late-summer temperatures, and higher elevation sites are more sensitive than low elevation ones to variations in this

climatic factor (Wilson et al., 2017). Therefore, latewood density chronologies can be used to discriminate trees per elevation in a first step, and then use ring-width chronologies to assign provenances within those elevations. Currently, latewood density can be derived from blue intensity (BI) measurements, as the blue light is absorbed by organic compounds related to cell wall thickness, namely hemicellulose, cellulose and lignin (Campbell et al., 2007, 2011; Wilson et al., 2017). BI is implemented in several dendrochronological software packages, where it can be measured simultaneously with ring-/earlywood/latewood-width. The systematic production of latewood density chronologies should therefore become the standard when researching conifers (living trees and historic timbers).

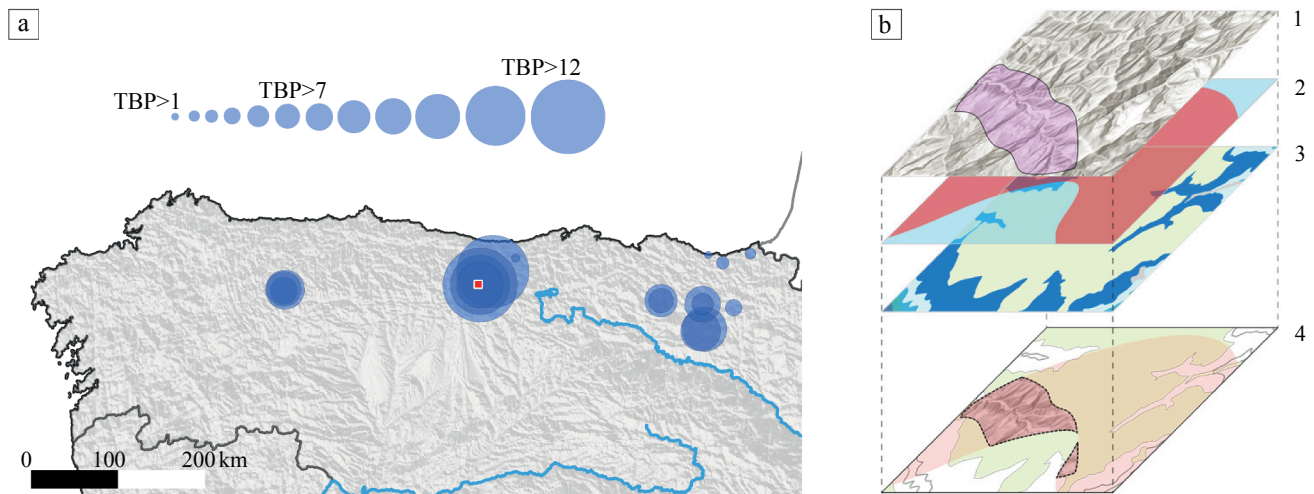
## 7. Geographic information systems as integration tool

The integration of results from the different aforementioned methods (ring-width, vessel-size, latewood-width and latewood-density BI chronologies, Strontium isotopes and organic compounds) should ultimately be combined in and linked to the geographical locations or areas where trees were harvested for construction purposes. A geographic information system (GIS) is therefore an essential tool to merge and visualize the provenancing results in georeferenced maps. This approach is particularly strong when combining results from wood studies (or other disciplines) with historical data linked to geographical locations, such as shipbuilding areas, potential sources of timber upstream from watersheds, forests reserved for naval timber production, etc.

Map layers of correlations between tree-rings of a sample and different ring width chronologies could be spatially interpolated, going from a point cloud indicating different correlations (the highest being the probable source of the wood) (Fig. 10a), to a spatially explicit probability of provenance layer (Fig. 10b). Other proxies from wood (Sr ratios, vessel size, latewood density variations, etc.) could then be used to help improve accuracy. Land-use or forest-use types (current and historical) should be included in these chronology databases, as trees within a type of forested landscape (closed forest, parkland, wooded pasture) may show stronger correlations over large distances than with nearby trees from a different forest type (Bridge, 2012). Combining GIS layers derived from climate-growth correlations with niche modelling (e.g., Vesella and Schirone, 2013; Ülker et al., 2018) could allow for the reconstruction of the potential current and past spatial distributions of the timber species. To effectively apply this method in the Iberian Peninsula, we encourage the sharing of existing data (e.g. Domínguez-Delmás et al., 2015; Susperregi and Jansma, 2017; Gazol et al., 2018; Akhmetzyanov et al., 2019; Akhmetzyanov et al., 2020) and extending retrospectively the chronologies compiled in Gazol et al. (2018) with historic timbers from buildings to create a network of reference tree-ring chronologies covering the Early Modern Period. Currently, the number and spatial coverage of long-span chronologies limits the possibilities of interpolating correlation values to create the probability layers of provenance for historical timbers. In the short-term, methodological advancements and tests of the viability of these integration tools could be carried out in locations with a high spatial coverage of chronologies (e.g. Central Europe, Scandinavia, Western United States).

## 8. Future steps towards the improvement of shipwreck timber provenancing methods

Our research has established a foundation for provenance studies of shipwreck timbers in the Iberian Peninsula, but there is still a long way to go. From the dendrochronological research we learned that finding forests that supplied oak timber for shipbuilding in the north of Spain is difficult, as those areas are now mostly depleted or covered by exogenous fast-grown species of economic interest (e.g. *Eucalyptus* sp.); furthermore, oak trees that regenerated locally are not only young, but also probably growing under considerably different climatic constraints compared to the past. Adding to this challenge is the fact that old trees



**Fig. 10.** a) Classic approach to dendroprovenancing, in which correlations between a tree-ring series, an object chronology or a site chronology (in this case ONQ chronology) are plotted as bubbles representing  $t$ -values (in this case expressed as TBP, Baillie and Pilcher, 1973), where the bigger bubbles indicate stronger statistical agreement; b) theoretical representation of a GIS model combining 1) dendrochronological results that point towards a specific elevation within a specific valley, 2) distribution map of species under study (in red); and 3) bioavailable strontium isoscape layer, to provide spatial probability of provenance in a map layer, represented in 4) by the area enclosed within the dash lines.

found in woodlands further inland have undergone intensive anthropogenic alterations (e.g. cycles of pruning for firewood), and they often present wounds, where the growth pattern is distorted, and rotten parts that result in hollow stems. Despite all these challenges, we managed to find old trees that have provided invaluable data, linking the present with the Age of Discovery (oaks in the north) and the late Middle Ages (pines in the south). These datasets can serve to establish benchmark provenancing statistical metrics for these regions, and to test novel statistical approaches for provenancing such as those proposed by Drake (2018), Boswijk and Fowler (2019), and Bridge and Fowler (2019).

The datasets produced within our project should be further expanded geographically as well as chronologically, using more forest sites, and timbers from numerous historic buildings. In this way, the lack of data from the original supply areas might be compensated by the collection of a large amount of data in nearby sites, so that a dense network of long-span reference chronologies can be developed and combined into regional chronologies. Even if they have relatively low geographical resolution, such chronologies could help establish absolute dates and provenances for archaeological wood originating in the Iberian Peninsula, as demonstrated for example by the results of the *Magdalena* shipwreck samples. Furthermore, a great number of local chronologies developed from numerous individual trees would contribute to minimize the effect forest practices, usually present as asynchronic disturbances within the tree-ring patterns. The development of vessel oak chronologies is also encouraged to counterpart the effect of natural and anthropic disturbances. Since they can also be obtained from historical and archaeological material, such chronologies have the potential to cover multi-century periods in the north of Spain (multi-millennia in central Europe). Sources of archaeological timber should also be sought in maritime structures from former centuries, as wood preserved in coastal, riverine, and lacustrine environments could also provide valuable data to prolong or improve the reference chronologies.

When it comes to isotopes, the strontium results underline the potential and importance of a new method combining  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{88}\text{Sr}$  values for provenance studies on wood or other materials, but they also highlight its limitations in some contexts. The reliability of Sr isotopes as a tracer of the provenance of archaeological material hinges on: i) measurable isotopic signatures characteristic of distinct geographical areas; ii) sufficient homogeneity within these areas; iii) isotopic signatures of biological materials that reflect those of underlying rocks or

soils; iv) limited diagenetic modification of the archaeological sample from its storage and/or depositional contexts; and last but not least, v) a specific protocol to eliminate contamination and identify the sample's initial Sr in order to allow accurate conclusions about its provenance.

Similarly, the use of FTIR and Py-GC-MS for wood provenance studies comes across important challenges when applied to shipwreck timbers, as the degradation processes that take place during centuries of underwater conditions must be understood and accounted for with each sample. Furthermore, the occurrence of potential hybrids within deciduous oak species is apparently common in the north of Spain (see [www.floraiberica.es](http://www.floraiberica.es)), and can represent a major challenge when trying to separate species based on organic components of the wood. Nonetheless, these techniques have great potential to complement dendrochronological and isotopic information. Future research should consider additional statistical approaches to explore the potential of applying multi-proxy measurements, and further sampling and analysis of reference material should be carried out for a more comprehensive and robust understanding of the results.

Additional approaches to timber provenancing should continue to be explored. Using xylem anatomical variables combined with ring-width measurements has the potential to improve the precision of dendroprovenancing, as proposed by Akhmetzyanov et al. (2019) in oaks and Akhmetzyanov et al. (2020) in pines. The result of those approaches is not influenced by the waterlogged condition of the wood, and could therefore be implemented on archaeological material. In pines, variation of wood biometry has been found to be higher and more pronounced among provenances than within populations for a given pine species (Esteban et al., 2012), and maximum latewood density of *Pinus sylvestris* has shown potential to improve the dating of historical samples (Wilson et al., 2017). Whereas the measurement of BI is straightforward, and should be considered as a rule, measuring and generating earlywood vessel chronologies is more time-consuming, and has therefore limitations to be applied to each single series. However, new possibilities for using quantitative wood-anatomical variables are currently available through the development of new tools, such as the core-microtome designed by Gärtner and Nievergelt (2010) for surface preparation of full increment cores, and the improvement of image analyses procedures (Von Arx and Carrer, 2014), including the potential application of newly-developed machine learning methods (De Mil et al., 2018).

## 9. Final comments

This paper illustrates the potential and limitations of provenancing wood in a worst-case scenario. Historic shipwrecks can seldom be positively identified; therefore, the premise that they may not originate from the area where they were found must remain at the forefront of the research. Additionally, they may have been built with wood from different (and sometimes very distant) areas, as commercial networks in the Early Modern Period connected woodlands with shipyards all over Europe and beyond (e.g. De Vries and Van der Woude, 1997; Crespo Solana, 2015; Kumar, 2018), which adds to the complexity of the research. Furthermore, even when the shipwrecks are identified, and historical archives reveal the sources of the timber (such as in the case of the *Magdalena* frigate), it is impossible to know which timber in the ship came from which exact area. Therefore, provenancing methods must be developed based on objective empirical tests, and applied blindly to the samples.

The methods presented are applicable in studying shipbuilding and timber supply in different periods and geographical regions beyond the Iberian case study discussed here. Continued and expanded analyses of ship timbers should furnish improved insights on former woodlands, the exploitation of their resources for shipbuilding, and the chronology and evolution of ship designs. A better understanding of the human impact on ecology and the repercussions of technological innovations throughout history contribute to debates about the definition and chronology of the Anthropocene (Trouet et al., 2017), providing empirical data about changes and developments that had incalculable impacts on past societies, and which continue to reverberate into our lives today and those of the future.

## CRediT authorship contribution statement

**Marta Domínguez-Delmás:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization. **Sara Rich:** Investigation, Resources, Data curation, Writing - original draft. **Mohamed Traoré:** Methodology, Formal analysis, Investigation, Validation, Resources, Data curation, Writing - original draft, Visualization. **Fadi Hajj:** Methodology, Formal analysis, Investigation, Validation, Resources, Data curation. **Anne Poszwa:** Conceptualization, Validation, Resources, Writing - original draft, Supervision, Project administration, Visualization. **Linar Akhmetzyanov:** Methodology, Formal analysis, Investigation, Resources. **Ignacio García-González:** Conceptualization, Methodology, Resources, Formal analysis, Supervision. **Peter Groenendijk:** Formal analysis, Resources, Data curation, Writing - original draft, Visualization, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Research data

Tree-ring datasets presented will be made available through a Data in Brief publication. Data resulting from the organic chemistry analyses has been published in Data in Brief: <https://doi.org/10.1016/j.dib.2018.11.032>.

## In memoriam

Fadi Hajj passed away too soon, in January 2018, just two months after defending his Ph.D. and when this manuscript was at an early stage. The section "Strontium isotopes as wood provenance markers" comes from original results that Fadi obtained during his PhD. Fadi was a brilliant young researcher, extremely kind, funny and caring, and a very good friend. Words fall short, but we hope that this contribution honours his memory. He will be forever present in our minds and hearts.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2020.102640>.

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