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Start date: August 2014

Project title: Application of ecological wood anatomy for species determination and wood provenancing of oak and pine from Atlantic Iberia (WP3)

Supervisor name: Ute Sass-Klaassen (WU)

Objectives

(1) Determination of wood-anatomical characteristics to enhance precision of wood provenancing by providing information on species detection, micro-climate, specific site conditions and past forest management

(2) Identification of anatomical biomarkers to determine the provenance of wood used in Atlantic Iberia for shipbuilding

Tasks and methodology

(1) Sampling of living oaks and pines from selected sites at Atlantic Iberia

(2) Analysis of quantitative and qualitative wood-anatomical characteristics of living oak and pines to identify anatomical biomarkers for oak and pines species for high-precision dendroprovenancing

Results/deliverables

Tree-ring database including records on wood-anatomical characteristics of oak and pine species from Atlantic Iberia reaching back to at least the 15th century.

Survey activities and milestones reached during period 15 august 2014- 31 august 2016

This progress report is based on the objectives and deliverables as proposed for the ForSEAdiscovery project which are elaborated specified in more detailed in the Ph.D. proposal submitted to the research school. (appendix 1).

Literature review on the potential of using wood-anatomical features to increase the precision of dendroprovenancing with special focus on maritime archaeology

Related to objective 1, status: **in progress**

The paper summarises an analyses of research based on time series of wood-anatomical features. Yet, this research was mainly focussed on methodological issues and improving the temporal resolution of climate growth relationships. We use results and insights from past studies to extract information on the potential of using times-series of easy measurable wood-anatomical variables for dendroprovenancing with focus on ring-porous oak species (*Quercus robur, Q. petraea, Q. pyrenaica, Q. faginea*) and pines (*Pinus sylvestris, P. nigra*). The paper contains a table as survey on all published studies. The paper will be submitted by the end of 2016.

Development of earlywood-vessel chronologies of ring-porous oak species from Atlantic Iberia

Related to objective 1 & 2, main deliverable; status in progress, table 1 for state-of the art

During two sampling campaigns (in May and October 2015) and we collected samples from more than 120 oak trees from seven sites in Northern Spain (fig. 1).

This material will be supplemented by a campaign, planned during September 2016, where 2 sites with oak will be sampled to cover the required longitudinal gradient (Asturias and Cantabria regions of Spain). Up to now, tree-ring width chronologies for oak are created for each study site (together with ESR 9 Marta Domínguez Delmás and ER3 Peter Groenendijk). Furthermore, earlywood vessel chronologies have been constructed for four study sites (fig. 2). Currently the material from oaks of the remaining five sites is analysed. The results will published in a peer-reviewed paper with the tentative title: *Analysis of climatic signal stored in oak vessels across Northern Spain* (planned for December 2016).

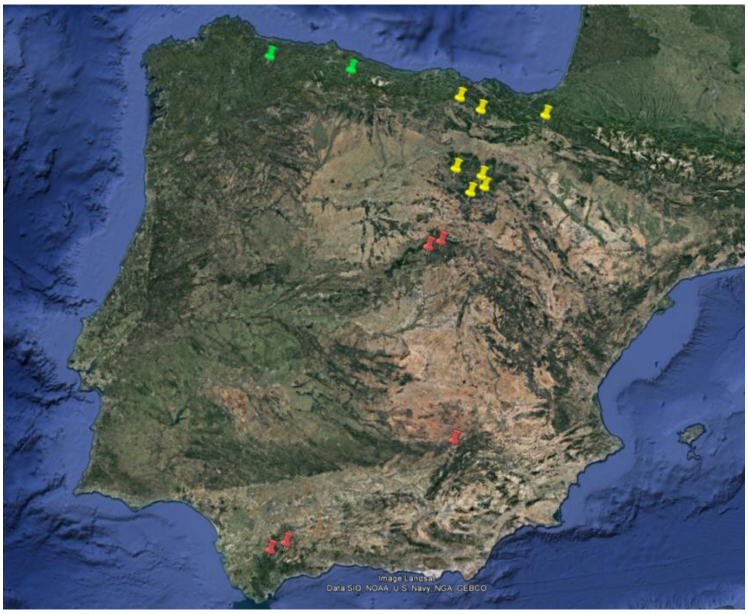

Figure 1: Sampling location of oak (yellow), pine (red) and future sampling areas for oak (green) oaks sampling areas

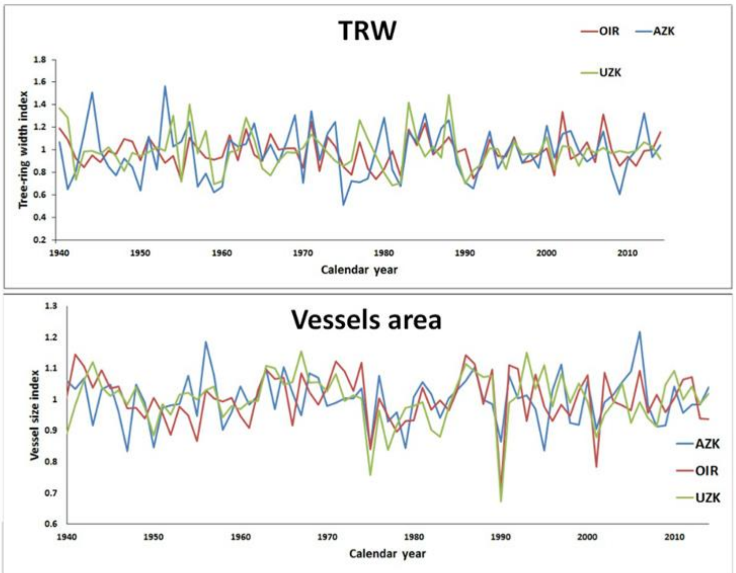

Figure 2: Example of recent part of tree-ring width (top) and vessel area (below) chronologies constructed for three oak sites in Iberia.

Development of maximum latewood density (MLD) chronologies of pines as a tool for dendroprovenancing

Related to objective 1 & 2, main deliverable; status in progress, table 1 for state-of the art

During three sampling campaigns we collected more than 200 pine trees from 5 sites in Central and Southern Spain (Fig. 1). The last sampling campaign for collecting material from two additional sites is scheduled for October 2016. Maximum latewood density has proved to be a meaningful wood-anatomical variable to assess climate-growth relations of coniferous tree species. All the collected material is stored in the laboratory of University de Santiago de Compostela. Tree-ring measurements have been conducted. During the last year we tested a new approach, blue-intensity measurements to efficiently detect this variable from carefully prepared wood cores. We succeeded in optimizing the preparation method and will apply the blue intensity technique to assess maximum density for all our study material. These analyses are planned to be done in the first half of 2017. Besides deriving long chronologies for dendroprovenancing the results on climate-growth analyses will be published in a peer-reviewed paper (Tentative title: *Maximum latewood density indicates origin of Iberian pine)* we will describe the potential of this wood-anatomical variable for dendroprovenancing of Iberian pines.

Application of DNA analyses for provenancing oak timber

Related to objective 2, in progress

Extracting genetic material from wood is still a challenge. Yet there is no standard protocol available for extraction of genetic information from – specifically – heartwood of fresh, historic and archaeological material. In a pilot study we assessed the amount of DNA which can be extracted from different wood compartments. i.e. bark & cambium, sapwood, heartwood (of fresh and historic timber). For this analyses, material of five oak trees has been collected and further used as a trial for extraction of genetic material from different parts of the tree (leaves, bark/cambium, sapwood and heartwood). The results of extraction were promising and needed minor corrections. Further analyses in this direction will be done in the second half of 2017. The aim is to use genetic information for (1) species determination (distinguishing between European (*Q. robur, Q. petraea)* and Iberian (*Q. pyrenaica, Q. faginea)* oak species) and (2) provenancing based on oak haplotypes. We expect to come up with a protocol for DNA extraction from heartwood of historic material and possibilities of DNA extraction from archaeological ship timber. The results will be published as a technical paper.

Tracing the impact of forest management on the application of wood-anatomical variables as tool for provenancing

Related to objective 2, planned for 2017

The application of chronologies derived from wood-anatomical variables for dendroprovenancing requires insight into environmental factors other than climate which impact tree growth and wood structure. An important factor is forest management, specifically in forest areas which are known to be as source areas for e.g. timber used for shipbuilding. The question arises whether forest management can obscure the information in chronologies of wood-anatomical variables and hence their value for dendroprovenancing. To tackle this research question, we will compare wood characteristics of oaks from managed and unmanaged sites. The hypothesis is that there is no significant effect of forest management on size of oak vessels. Planned article will be done in parallel to the main work about the oak vessels from Northern Spain.

Case study to verify the added value of Maximum latewood density for precise provenancing

Related to objective 2, planned for 2017

For this case study we will use wood from an already dated (by using tree rings and verified by historic documents, by ESR 9) historic building, the Segovia cathedral. We will test (1) whether the blue intensity technique will work on historic material and (2) if provenancing using various variables (anatomical variables, tree rings (ESR9) and chemical composition (ESRs 11 and 12) based on the collected network of site chronologies and wood composition of trees from this sites (chemical traits) will lead to comparable results.

Table 1. Progress timetable of Akhmetzyanov Linar/ESR10

Chapter / Progress	Samples/Data collection			Samples preparation			Tree-ring width measurements			Anatomical measurements			Data Analyses			Publication
Added value of wood-anatomical features for dendroprovenancing				N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A				December 2016
Oak vessels as a tool for dendroprovenancing.																December 2016
Maximum latewood density (MLD) of pines as a tool for dendroprovenancing.																June 2017
Genetic analyses of wood for species identification.							N/A	N/A	N/A	N/A	N/A	N/A				December 2017

ForSEAdiscovery – events/activities attended/organized

Event Number dived in Intern=ForSEAdiscovery events and extern= events with benefit for ForSEAdiscovery	Participant hosting the event	Type of Event	Month when the event took place	Start date of the event	End date of the event	Total number of researchers outside the network attending the event	Personal activity	Website of the event
Intern 1	Partner: University of Santiago de Compostela	Sampling campaign (*Pinus nigra),* Andalusia, Spain	November 2014	22.11.2014 -	27.11.2014	only WP3 ESRs	Sampling	N/A
Intern 2	Partner: Centro de Ciencias Humanas y Sociales (CCHS) del Consejo Superior de Investigaciones Científicas (CSIC)	ForSEAdiscovery Project network meeting	January 2015	12.01.2015	13.010.2015	Project members	Presentation	http://forseadiscovery.eu/
Intern 3	Partner: Centro de Ciencias Humanas y Sociales (CCHS) del Consejo Superior de Investigaciones Científicas (CSIC)	ForSEAdiscovery: INTENSIVE TRAINING COURSE: History of Wooden Shipbuilding & Books and treatises on Shipbuilding:	January 2015	14.01.2015	16.01.2015	all ESRs	Attendance	http://forseadiscovery.cchs.csic.es/courses/history-wooden-shipbuilding-books-and-treatises-shipbuilding

Intern 4	Partner: Archeonauta S.L , University of Santiago de Compostela, Wageningen University	Sampling campaign ship wreck, Cádiz	February 2015	9.02.2015	12.02.2015	ESRs 9, 10, partner Archeonauta S.L	Sampling	N/A
Intern 5	Partner: University of Santiago de Compostela	Sampling campaign (*Quercus spp.),* Basque country, Spain	May 2015	11.05.2015	31.05.2015	only WP3 ESRs and ER	Sampling	N/A
Intern 6	Partner: University of Santiago de Compostela	Sampling campaign (*Quercus spp.),* Basque country, Spain	October 2015	05.10.2015	12.10.2015	only WP3 ESRs and ER	Sampling	N/A
Intern 7	Centro de Ciencias Humanas y Sociales (CCHS) del Consejo Superior de Investigaciones Científicas (CSIC)	ForSEAdiscovery: INTENSIVE TRAINING COURSE: Application of Geographic Information System (GIS) to Maritime History and Archaeology	October 2015	13.10.2015	16.10.2015	all ESRs and ERs	Attendance	http://forseadiscovery.cchs.csic.es/courses/application-geographic-information-system-gis-maritime-history-and-archaelogy
Intern 8	Partner: Wageningen University	ForSEAdiscovery: INTENSIVE TRAINING COURSE: Dendrochronology and Wood anatomy	December 2015	09.12.2015	11.12.2015	all ESRs and ERs	Organization of the Workshop; Presentation “Application of wood-anatomical analyses for dendroprovenancing”	http://forseadiscovery.cchs.csic.es/sites/default/files/attachments/documents/workshop_dendrochronology_wur.pdf

Intern 9	Centro de Ciencias Humanas y Sociales (CCHS) del Consejo Superior de Investigaciones Científicas (CSIC)	ForSEAdiscovery Midterm Project network meeting	December 2015	15.12.2015	18.12.2015	Project members	Presentation	http://forseadiscovery.eu/
Intern 10	Partner: University of Santiago de Compostela	Sampling campaign (*Pinus sylvestris.),* Central System, Spain	July 2016	11.07.2016	15.07.2016	only WP3 ESRs and ER	Sampling	N/A
Extern 1	Partner: University of Santiago de Compostela	European Dendroecological Fieldweek, Oviedo, Spain	September 2014	01.09.2014	06.09.2014	c. 30	Presentation	http://www.cetemas.es/post/fw14-flyer-european-dendroecological-fieldweek-2014.pdf
Extern2	Partner: University of Santiago de Compostela	EuroDendro 2014, Lugo, Spain	September 2014	08.09.2014	12.09.2014	c. 100	Poster presentation	http://www.usc.es/en/congresos/eurodendro
Extern 3	Partner: University of Wageningen	Basic Statistics	October 2014	13.10.2014	17.10.2014	c. 20	Attendance	N/A
Extern 4	COST training school; Partner: University of Wageningen	Training School: Quantitative Wood Anatomy: from sample to Data, Zurich, Switzerland	November 2014	18.11.2014	21.11.2014	c. 20	Attendance	http://streess-cost.eu/streess-news/63-news/319-upcoming-training-school-2
Extern 5	Partner: University of Wageningen	Wood under Spotlight – Presentation for students at Wageningen University (presentation, M. Dominguez, U. Sass-Klaassen, L. Akhmetzyanov)	February 2015	17.2.2015	17.2.2015	c. 100	Organization of the event	http://www.wageningenur.nl/en/activity/Wood-in-the-SPOTlight-Interactive-evening-on-wood-its-science-and-beauty.htm
Extern 6	Partner: University of Wageningen	The Essentials of Scientific Writing and Presenting	March 2015	09.03.2015	14.03.2015	c. 20	Attendance	N/A

Extern 7	COST training school; Partner: University of Wageningen	Statistics in Dendrochronology	March 2016	14.03.2016	16.03.2016	c. 20	Attendance	N/A
Extern 8	COST training school; Partner: University of Wageningen	Final COST STReESS meeting, Joachimsthal (Berlin), Germany	April 2016	13.04.2016	14.04.2016	c. 100	Poster	http://streess-cost.eu/index.php/events/meetings/final-meeting
Extern 9	University of Wageningen	Supervision	May 2015 - ...	27.05.2015	Now	1	Supervision of Master student	N/A

Appendix 1. Research proposal

Title: Application of ecological wood anatomy for species determination and wood provenancing of oak and pine from Atlantic Iberia (WP3)

1. SUMMARY (max 250 words)

Unprecedented demands on Iberian oak and pine forests led by increase of ships building in the Age of Discovery (16th to 18th century) caused intensive forest logging practices on the Iberian Peninsula. However, it still remains unknown if Iberian forests could sustain this increasing demand or whether timber was imported from elsewhere. In addition, the exact origin of the growth of the timber used for shipbuilding in Iberian Peninsula during that time is also not identified yet.

This project aims to study xylem structure of Iberian pine and oak species, intensively used for shipbuilding, to add to already existing time-series and to use them for provenancing of ship timber. I will study earlywood vessels from five oak species from Northern Spain and tracheid dimensions together with maximum latewood density of two pine species from Central and Southern Spain. State-of-the-art approaches will be used to build long time-series from anatomical features and to compute site specific wood-anatomical markers for each study site. Analyses of ship timber will be carried out following the same approach. Provenancing will be done by statistic comparison of time series gained from ship timber with existing and newly developed reference records built up in this study. In addition, samples from living trees and shipwrecks will undergo genetic analyses in order to identify species used for specific parts of the ships.

This study will be among the first using xylem features for provenancing purposes, and will provide substantial input for dendroprovenancing studies.

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2. DETAILED DESCRIPTION OF RESEARCH AREA

Introduction

During the Age of Discovery and European Expansion (16th to 18th centuries), ocean-going ships became paramount to the development and sustainability of Iberian empires. The construction of ships placed unprecedented demands on Iberian forests, leading to **forest management practices** and enactment of laws to ensure **timber supply for shipbuilding** [1, 2].

Oak and pine were the preferred species for structural timbers of ships, hence they can be found nowadays in **shipwrecks** from Spanish and Portuguese ships in different parts of the world [3]. However, the identification of those ships by name and nationality is problematic if not impossible, as the establishment of their exact place and time of construction remains a challenge for historians, nautical archaeologists and dendrochronologists [4].

The interdisciplinary Marie Curie ITN ForSEAdiscovery project combines disciplines from the Humanities (historiography and nautical archaeology) and the Life Sciences (dendrochronology, wood anatomy, and geo/dendro-chemistry) to address the broad question of the timber supply for Iberian Empires in the Age of Discovery (16th to 18th century). A specific question for the Life Sciences group is the development of **methods for provenancing i.e. identification of the geographical origin of the growth of oak and pine ship timbers derived from trees grown in the Iberian Peninsula.** Establishing the Iberian origin of timbers retrieved from shipwrecks would inform about the extent of the timber provision from specific Iberian forests during the 16th to the 18th century, and the **changes in forest structure and management** as a consequence of exploitation and further degradation. In this manner, wood recovered from shipwrecks represents a natural archive to reconstruct forest history and ancient management practices [3].

At present, provenancing Iberian ship timber is hampered by the lack of reference datasets for the peninsula (e.g., a wide-coverage tree-ring data network spanning more than 500 years). The development of **identification keys based on genetic and wood-anatomical features** (i.e. independent from ring-width datasets) to identify oak and pine timber as "Iberian", would be a major contribution to the much needed reference data network. For this, we propose to study **wood-anatomical characteristics** determined by i) genetics of the species, ii) local/regional growing conditions (forest ecology and regional climate), and iii) forest structure and ancient forest management practices, which result in specific timber qualities. We propose to study wood anatomical features by analyses of scanned images of the tree cores and shipwrecks. We anticipate that the methods developed could be employed to provenance timber elsewhere.

Identification of Iberian oak and pine species

Several oak species used in shipbuilding in Atlantic Iberia are endemic to the Iberian Peninsula and North Africa (e.g. *Quercus pyrenaica* and *Q. faginea*). However, their identification based on wood-anatomical features is not yet possible, as their wood anatomy does not differ from those of other European oaks also present in the peninsula (*Q. robur, Q. petraea, Q. pubescens*) [5]. Genetic markers could serve as tool for species identification [6, 7] provided that DNA fragments can be extracted from the wood of Iberian oak and pine wood [8, 9] and specifically from ancient ship timber [10, 11]. Recent studies demonstrated the feasibility of DNA barcoding techniques to identify tree species using archaeological timber [12]. And belonging of *Q. pyrenaica* to different section (*Mesobalanus)* supports application of DNA barcoding technique for its discrimination from other oak species widely used for shipbuilding. However, if DNA extraction from leaves is straightforward, DNA extraction from dried wood as well as wood stored for a long-term, is more problematic as wood DNA becomes seriously degraded and the succession rate (rate between number of samples with successful extraction of DNA material and total number of samples used for analyses) still remains low [13-15].

Tools for dating and dendroprovenancing

Dating and provenancing ship timber can be achieved by applying classical dendroprovenancing, based on ring-width analyses [16, 17] . This implies that the ring-width series of a given ship timber is compared to time-series from a network of regional and local chronologies from the same species. Further, the chronology yielding the highest correlation (e.g. t-test (e.g. [18]), coefficient of parallel variation "Gleichläufigkeit" [19]) at a certain position serves as an indicator of the cutting date and geographical origin of the tree used as timber [16]. This method has been successfully applied in many studies [18, 20-22], particularly on ship timber [23-25]. However, the lack of a reference tree-ring data network for the main supplying areas of the Iberian Peninsula, hampers the application of this method when researching Iberian ship timbers. Therefore, extension of the area covered by tree-ring width chronologies is strongly required for provenancing of Iberian shipwrecks.

The spatial precision of provenancing provided by annual variation in ring-width patterns can potentially be further enhanced by including additional information stored in the anatomy of the wood. The field of quantitative wood anatomy has strongly developed in the last years due to the improvement of measurement equipment, which allows measuring wood-anatomical features with high precision and efficiency [26-28]. Wood-anatomical features hold information on site-specific, mainly climate forced, growing conditions, with the potential of being valuable indicators for provenancing. For instance, features that can be considered as regional and/or site-specific markers comprise **quantitative continuous anatomical characteristics**, such as annual variation in earlywood vessel area in oak species [29-33] and density features in pines [34-36], as well as **qualitative, discrete anatomical characteristics** such as intra-annual density variations, e.g. "light rings" in pines [37-41].

Besides the site-specific conditions, **long-term variations in ring-width patterns** contain information on specific growth trajectories of a tree population with abrupt changes being an indicator of natural or man-made (selective cutting campaigns, tending operations, coppicing) disturbances [42, 43]. Management activities leave specific traces in time series of ring-width (e.g. growth releases due to harvesting, selective cutting, thinning) and wood anatomical variables (e.g. changes in vessel patterns in resprouts from coppicing) [44, 45]. This allows detecting past management activities but can lead at the same time to a distortion of the regional climate signal in the ring-width series, hampering dendrochronological crossdating.

Sample collection on oak and pine species will be organised in joined field campaigns (forests and shipwrecks) with other project partners. The study material for this study includes i) living trees from Iberian oak and pine species growing in forest known to be source areas for Iberian ship timber during the Age of Discovery, ii) archaeological wood from timbers of shipwrecks suspected to be of Iberian origin, and iii) wood from historical buildings that will serve to link the ring-width and wood anatomical proxies from the living trees and the archaeological timbers.

Objectives

This study is executed in the context of the ForSEAdiscovery project. One of the main goals of the ForSEAdiscovery is to provenance the timber used for shipbuilding in Iberian Peninsula during the Age of Discovery (16^{th} to 18^{th} century). The overall objective of this PhD study is to use anatomical structure of the xylem for provenancing the timber from shipwrecks. The specific objectives of this Ph.D. project within the ForSEAdiscovery are:

(1) To identify oak species from shipwrecks of presumably Iberian ships by applying genetic techniques.
(2) To analyse and quantify the relationship between site conditions and growth as reflected in xylem-anatomy of Iberian oak and pine species and thus to develop site specific anatomical markers.
(3) To provenance oak and pine timber used for ship construction by Iberian empires in the age of Discovery by applying wood – anatomical analyses and application of the markers developed under (2) to identify geographically distinct population.

Research questions and hypotheses

Research question 1. Can genetic material be extracted from oak wood and used as a tool for identification of Iberian oak species?
We hypothesize that genetic analyses will result in reliable identification of oak species endemic to Iberian peninsula (*Q. faginea* and *Q. pyrenaica)* and of oak species with a wider distribution across Europe (*Q. robur, Q. petraea, Q. pubescens*). However, discrimination between deciduous oaks species (*Q. robur/ petraea/ faginea/ pyrenaica/ pubescens*) based on wood anatomy is not feasible. Potentially identified specific markers for the Iberian species will also be tested if and how they can be extracted from ancient wood (ship timber).

Research question 2.1.Can time series derived from earlywood vessel size of Iberian oak species serve as wood-anatomical marker for provenancing of oak timber? We hypothesize that earlywood vessels of Iberian oak species are more sensitive to growing conditions than tree-ring width series.

Time series of earlywood-vessel area contain a strong environmental and climatic signal that provides highly accurate information [29-31, 46]. Recent studies on early-wood vessel series of deciduous oak trees from Northern Spain (Fig.1) proved the strong effect of - especially early spring - precipitation on earlywood-vessel area of *Q. robur* [29] and of precipitation along the previous growing season on earlywood-vessel area of *Q. pyrenaica* [32]. In addition, the area of the first row of vessels also reflects precipitation amount at the beginning of the growing season [31]. This dataset will be accomplished by vessel chronologies derived from living oaks and historic oak timber with known (local) origin. The latter is necessary to prolong vessel chronologies back in time.

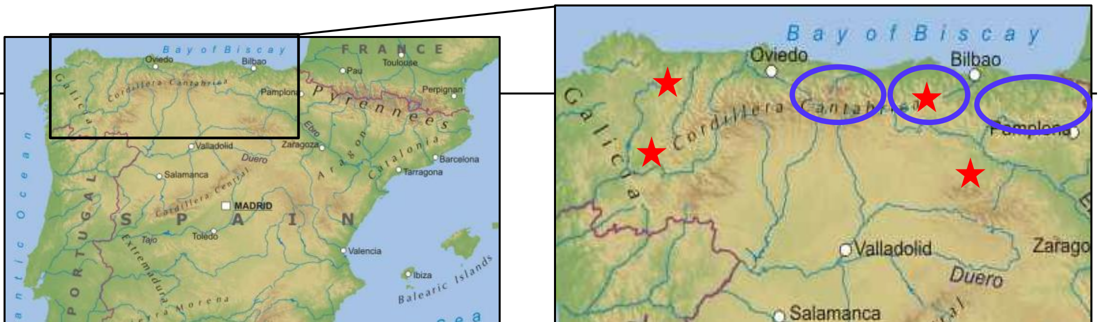

Fig.1. Map of already existing oak earlywood vessels chronologies in Northern Spain (stars) and oak sample areas for this study (circles).

Research question 2.2. Can high-resolution wood anatomical variables (maximum latewood density and tracheid dimensions) derived from tree rings of Iberian black pine and Scots pine serve as ecological markers to enhance the precision of provenancing for pine timber? We hypothesize that wood anatomical variables of Iberian pine species contain more site specific information about the growing conditions than tree-ring width series.

The most prominent wood-anatomical variables extracted from tree rings of coniferous species are related to tree-ring density and comprise maximum latewood density (MLD) [47, 48], and minimum density or light rings (MD) [41, 49]. In addition, intra-annual density variations (IADFs) have been identified as high-precision markers to assess fluctuations in climate parameters during the growing season [37, 38]. Maximum density of tree rings from pine trees has shown to contain a stronger climate signal than ring width [35, 36] and has been used as proxy for drought in arid areas [50]. Minimum density (MD) (light rings) may occur as a result of low temperatures during growing season at subarctic treeline [41] as well as severe drought from previous autumn to summer of the current year in semi-arid areas [51].

Besides density variables, the tracheid-lumen area contains strong environmental signals related to water availability across the growing season [35, 52, 53]. I hypothesize that site-specific unfavourable growing conditions in the beginning of the growing season will be reflected as smaller earlywood tracheid size in the tree-ring of the corresponding year due to strong dependency of tracheid size on water availability in early summer [53] and during the phase of cell formation [54]. Further, I expect higher MLD values as a reaction to warmer summer temperature [34, 35] while light rings (low MD) will serve as an indicator of low summer temperature.

Research question 2.3. Do trends in ring-width and earlywood vessel patterns of Iberian oak species show the footprint of management activities in the past? I hypothesize that the annual variation in earlywood-vessel area will not be affected by management practices such as coppicing or pollarding

Trees from managed forests are usually excluded from the classical dendroprovenancing approach, as their ring-width patterns are strongly influenced by the management activities, which camouflage the high-frequency variation derived from regional and site specific climatic factors. Among the strongest management-related impact is establishment of oak coppice systems with short rotation cycles, which result in fast-growing trees needed for specific ship parts, such as framing elements. This coppicing practice leads to short, complacent ring-width series which are often not datable [55]. However, the effect of coppicing/pollarding on wood-anatomical features is still poorly understood. Recent studies suggest that earlywood oak vessels retain a strong climate-related variation even when this is not reflected in the ring widths (García González, pers. comm.).

Research question 3. Does the timber used for ship building by Iberian e during the Age of Discovery has an Iberian origin? I hypothesize that the knowledge gained from research questions 1 to 2 will allow (1) species identification, through wood anatomy, DNA techniques, and (2) estimation of exact origin of the timber.

Depending on insights gained from research questions 1 and 2, different wood-anatomical markers will be measured on a selection of timbers extracted from at least two shipwrecks which - based on historical records (Working package 1) - were built in Iberia with Iberian oak and/or pine wood. Feasibility of using these analytical methods on archaeological ship timbers will be assessed.

Methods

Research question 1.

Samples. The sample assemblage will include increment cores from living Iberian oak trees (*Q. robur/petraea/pubescens/pyrenaica/faginea*) from Northern Spain (Basque country and Cantabria mountains) and oak ship timber from Atlantic coast of Iberian Peninsula (sample protocols to be agreed within EU-ForSEADiscoveries programme).

Analyses. Genetic analyses will be executed on samples from living trees (leaves, sapwood and heartwood) and from ancient wood (sapwood, heartwood, dried and waterlogged material) [56]. For DNA extraction we plan to compare different methods as CTAB (etyltrimethylammonium bromide), DNeasy Plant minikit (Qiagen) or PTB (N-phenacylthiazolium bromide) [12, 56]. In cooperation with specialised DNA laboratories we will further develop appropriate methods and we intent to apply both microsatellites (simple sequence repeats) for haplotype identification and appropriate DNA barcoding techniques for closely related species [11, 56, 57]

Research question 2.1.

Samples. Material will include: i) samples from living oaks from Northern Spain (Basque Country and Cantabria region); ii) oak timbers from buildings located in the vicinity of the valleys where the living trees will be sampled. Living oaks will be sampled from different elevations along the longitudinal gradient of Cantabrian mountains. Each chosen elevation will contain several sampling sites and from each site 20 dominant trees of one of the oak species under analyses (depending on presence) will be selected for sampling. All trees will be cored at 1.3m with 60 cm length 5 mm diameter Pressler increment borer.

Analyses. Measuring of earlywood and first-row vessels will be performed on oak samples with previously crossdated ring-width series. Ring-width analyses and crossdating is done in cooperation with Marta Domínguez Delmás, ForSEADiscoveries PhD at the Universidad de Santiago de Compostela. After crossdating, samples will be prepared for vessel measurement: by black staining of the wood surface and consecutive treatment with chalk or white wax to fill the vessel lumina and improve vessel detection. Image analyses will be carried out using *ImageProPlus* and *ROXAS* software [58] which are available in DendroLab of WUR. Time series of vessel variables will be calculated and crossdated. Relationships between all these variables and environmental factors will be studied to understand how they influence vessel size and quantity at each site. Site specific vessel-related anatomical markers will be detected using the software *Weiser* [59], which allows detecting pointer intervals and pointer values in tree-ring series.

Research question 2.2.

Samples. Living black and Scots pines will be sampled in two major mountain ranges: the Central System, in the centre of Spain, and the eastern Baeticas Mountains, in the south of Spain. Different sites will be selected following altitudinal gradients. Sampling strategy will be the same as in RQ 2.1 (20 dominant trees of one specie from each site). The first sampling was carried out in the Cazorla Mountains (Southern Spain) in November 2014. Historic pines from buildings in the vicinities of the selected forests will also be researched following the same methodology than for the living trees.

Analyses. Tracheid-size measurements across the tree ring will be done by ROXAS [58]. Tracheid trends across the tree ring will be analysed by using the partitioning approach (Carrer, not published), where the tree ring is split into an equal number of sectors and changes of tracheid sizes in different parts of the ring (associated to time approx. time of formation within the growing season) will be obtained. ROXAS has a special routine to perform these analyses. As a result, several tracheid time series will be obtained for each study plot. They will be correlated with environmental variables (temperature, precipitation, soil characteristics, etc.) to assess their relationships. Site-specific tracheid (profile) characteristics will be defined and their application for provenancing checked.

To efficiently determine MLD and MD for large tree-ring series of *Pinus sylvestris* and *P. nigra* the blue intensity (minimum blue intensity) approach will be applied. This technique provides an inexpensive, robust and reliable surrogate for maximum latewood density measured by radiodensitometric technics [60]. After extracting the resin from the samples using acetone, blue intensity will be measured using CooRecorder software (CDendro package). Afterwards, maximum and minimum latewood density chronologies will be computed for calculation of site specific climate-growth relationships and pointer-year analysis. Site specific anatomical markers derived from tracheid lumen area and density variables will be detected by using *Weiser* software [59]. In addition the study material will be inspected for presence and frequency of intra-annual density variation as an indicator of abrupt changes in growing conditions (summer drought or substantial increase in early autumn precipitation [37]).

Like in the case of oak, the resulting database of newly developed wood-anatomical chronologies for the two pine species will be integrated with existing data on these variables (e.g. the Mediterranean IADF database collected within the COST STReESS network [37, 38, 54]).

Research question 2.3.

Samples. The study will be conducted on trees from coppiced stands and neighbouring non-managed stands in two regions in Northern Spain, the Basque Country and the Cantabrian region. At least three pairs of oak stands (coppiced and non-managed) will be selected to test the hypothesis. Compass oak timber retrieved from wrecks of suspected Iberian ships will be analysed following the same steps and methods.

Analyses. The same approach described for *Question 2.1* will be used for computing earlywood-vessel chronologies which will subsequently be analysed for systematic differences between oaks from unmanaged and managed trees.

Research question 3.

Samples. Timber from shipwrecks will be provided by colleagues from Work package 2, ship archaeology of the ForSEAdiscovery project.

Analyses. The methods described in research questions 1 to 4 for DNA and wood-anatomical analyses will be tested and applied on waterlogged wood. Provenancing based on wood-anatomical variables will be done by statistic comparison (T-test, correlation, percentage of parallel variation, pointer-year analyses) of time series gained from ship timber with existing and newly developed reference records built up in this study.

Innovative aspects

This study is among the first ones where quantitative wood anatomy is integrated with history and nautical archaeology (social sciences) and organic and inorganic wood chemistry as well as classical tree-ring research (natural sciences) to be used for one specific research objective: wood provenancing.

Though application as a **tool for precise provenancing of (ship) timber** is the main goal of this study in the framework of the ForSEAdiscovery project (RQ 3), this project will provide new insights in **long-term site-specific climate-growth relationships** (RQ 2.1&2.2) for five important oak and two important pine species at the Iberian peninsula, a region to be largely effected by predicted climate change [61]. In this context the **effect of (past) forest management on tree growth (RQ 2.3)** is another interesting issue, which will be addressed in this study, but also in the ForSEAdiscovery project.

The **application of DNA techniques for species identification** (RQ 1) requires the development of a systematic approach to extract DNA from recent wood (living trees) and ancient wood (ship timber), which is still far from being a standard procedure. This will be done with support from colleagues from Alterra (NL), INRA (F) and the von Thünen Institute (D). Results from this European-timber tracing exercise could be of importance for ongoing research on tracking of the origin of tropical timber, which experiences similar methodological issues.

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Work program

Supervision

Supervision will be done by a multidisciplinary team with day-to-day supervision by Dr. Ute Sass-Klaassen (FEM, WUR). Dr. Ute Sass-Klaassen is heading the DendroLab at Wageningen University. She is an expert in dendrochronology and quantitative wood anatomy and has experience in dendroarchaeology, tree biology, and forest ecology. My promotor, Prof. Dr. Ir. G.M.J. Mohren (FEM, WUR), is an expert in forest ecology and management; he will supervise the study on a regular basis and act as the main promotor for the PhD dissertation.

The team is completed by Dr. Ir. Jan den Ouden (FEM, WUR), specialised in forest and species ecology and effects of forest management on tree growth. Dr. Ignacio García González (Universidad de Santiago de Compostela, Spain) is an experienced dendrochronologist and the leading expert in wood anatomical features and climate-growth relationships of Iberian deciduous oak species. He will provide advice on analyses of earlywood vessels and footprints of coppicing in anatomy of oak trees.

Evaluation of my research progress will be carried out during formal and informal meetings with my supervisors on almost monthly basis. Most of the supervisors are located in Wageningen UR and easily approachable and available. Contact with Dr. Ignacio García González will be kept via e-mails and Skype. In addition, I will have a daily contact with Dr. Ignacio García González during my secondment period at the Universidad de Santiago de Compostela. Moreover, the progress of the study will be presented to members of the FEM chair group and other relevant discussions and conferences.

Execution

Technical equipment and facilities to perform wood anatomical research are largely available at the DendroLab of Wageningen UR. For analyses of minimum blue intensity CDendro software package must be purchased.

Tentative content of the PhD thesis:

During the course of the project, a PhD thesis, for defence at Wageningen University, will be written in the form of several scientific papers, together with an introduction and a synthesis. The tentative content of the dissertation follows the structure of the objectives and research questions as outlined above and may consist of the following chapters:

1) Identification of oak species from shipwrecks through genetic techniques.

2) Site conditions and growth as reflected in xylem-anatomy of Iberian oak species.

3) Site conditions and growth as reflected in xylem-anatomy of Iberian pine species.

4) Origin of oak and pine timber used for ship construction by Iberian empires

3. SOCIETAL SIGNIFICANCE

The sustainable use of forest resources was and still is a relevant issue. In the 16th to 18th forest exploitation and forest management at the Iberian peninsula was largely affected by ship building. Assessment of the impact of non-sustainable exploitation of forest resources creates awareness on the long-term consequences on forest structure and landscape development.

The interdisciplinary context of this project allows to provide answers to actual discussions on man-environment interaction, application of DNA techniques for timber tracing, climate impact on tree growth of two important European tree genera, oak and pine and impact of past forest management on tree growth and wood quality.

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4. DATA MANAGEMENT

Data management will be done according to Data Management Plan of Forest Ecology and Forest Management Research Group (FEM). In addition, more comprehensive personal data management plan is developed. According to this plan: short term storage of data will be done on external drivers and hard drive of personal laptop; long term storage will be done on common data repository of FEM; data produced within this project will be property of FEM Research Group; data produced will be shared with other members of the ForSEAdiscovery project.