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Identification of natural sunken wood samples

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Abstract

Sunken woods are abundant in deep oceanic environments, housing a huge faunal diversity. Studies on that substrate firstly focused on the associated organisms, but since a few years, identification of wood is a further aim. The purpose is to appreciate its degradation state, diversity, geographical origin and to identify specific associations between colonizing organisms and substrates. The first determinations were made on sunken woods from Taiwan/Philippines, the Vanuatu Archipelago, and the Mediterranean Sea. Samples' identification was based on histological studies. Different preparation techniques were used, depending on their degradation state. Detailed anatomy descriptions were made and compared to the native flora and the introduced species. Wood samples were well preserved. Diversified species were encountered, seemingly originating from local floras. *In situ* settlements of known wood species will enhance the knowledge of degradation and colonization degrees. *To cite this article: M. Pailleret et al.*, *C. R. Palevol 6 (2007)*.

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Résumé

Identification d'échantillons de bois coulés naturels. Les bois coulés sont abondants dans le milieu marin profound et sont colonisés par une faune très diversifiée. Les recherches ont d'abord été axées sur les organismes associés, mais aujourd'hui, l'étude du substrat est une nouvelle approche, permettant d'apprécier l'état de dégradation des échantillons et d'effectuer leur identification. La diversité des essences présentes dans ces écosystèmes et leur origine géographique seront ainsi évaluées. D'éventuelles associations spécifiques entre organismes et bois seront mises en évidence. Les premiers bois coulés faisant l'objet d'une identification proviennent de Taiwan/Philippines, de Vanuatu et de la Méditerranée. Les techniques de préparation des échantillons ont été adaptées à leur état de dégradation. Leur détermination est basée sur une étude anatomique comparative avec la flore locale ou non native. La structure des bois étudiés était bien conservée. Diverses espèces ont été identifiées, originaires des flores locales. Des expériences in situ

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permettront l'étude approfondie des processus de dégradation et de colonisation des bois. *Pour citer cet article : M. Pailleret et al.*, *C. R. Palevol 6 (2007)*.

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Keywords: Sunken wood; First identifications; Local flora; Vanuatu; Taiwan/Philippines; Mediterranean sea

Mots clés : Bois coulés ; Premières identifications ; Flore locale ; Vanuatu ; Taiwan/Philippines ; Mer Méditerranée

1. Introduction

Sunken woods are debris (branches, trunks) that come from terrestrial woody plants. They are carried along rivers to the ocean, then they drift with marine currents, become saturated with water, and sink. They are present at all depths [25], but the study mainly focuses on samples recovered in deep oceanic environments. They house a huge faunal diversity (gastropods, polyplacophoran and bivalve molluscs, decapod and peracrid crustaceans, polychaetes and echinoderms) [25], closely related to the fauna encountered around hydrothermal vents, cold seeps, and whale falls [7.11].

However, sunken wood ecosystems remain poorly known compared with these other deep-sea ecosystems [19]. Studies on that substrate firstly focused on the associated organisms [12,20,21]. Animals gather around sunken woods to feed on it or to take shelter [25].

Since 2003 (beginning of BOA program, *Bois coulés et organismes associés*), botanical identification of wood is a further aim. The purpose is to appreciate its degradation state, diversity, and geographical origin, as well as to identify specific associations between colonizing organisms and substrates [16]. The first determinations, made on sunken woods from Taiwan/Philippines, the Vanuatu archipelago and the Mediterranean Sea, are presented here.

2. Material and methods

Nine sunken wood samples were recovered during several cruises:

- three samples recovered during MUSORSTOM 3 (June 1985) off the coast of the Philippines (195-m depth, CP 101, 14°00′N, 120°19′E), kept in alcohol and then in water;
- one sample recovered during TAIWAN 2002 (May 2002), off the southwestern coast of Taiwan (0–400-m depth) kept dry;

- three samples recovered during BOA 0 (November 2004) off the coast of the Vanuatu archipelago (one in Big Bay, CP 2313, 15°05′S, 166°55′E, 450-m depth, kept in glutaraldehyde; two near Epi, C2304, 16°37′S, 167°59′E, 570-m depth, kept in alcohol);
- two samples recovered in the Mediterranean Sea, off the coast of Banyuls-sur-Mer in October 2005 (27-m depth, 42°29′N, 03°08′E, kept in alcohol) and January 2006 (57-m depth, 42°29′N, 03°10′E, kept in alcohol).

Anatomical description and identification of each sample are based on the study of three classic sections: transverse, tangential longitudinal, and radial longitudinal. Four cellular types are observed: vessel elements, axial parenchyma, rays, and fibres.

Several techniques of sectioning are used, depending on the wood degradation state and expected observations. Soft samples are embedded in a polyester resin, cut with a diamond saw, dressed and pasted on a glass slide, and then abraded to have thin sections (thickness of 40 µm and less). Well-preserved samples are cut according to the three classic sections with a razor blade or a Reichert sliding microtome (thickness of 30 µm for transverse section and about 17.5 µm for longitudinal sections). Sections are commonly bleached, stained with iodine green, and mounted in gelatinous glycerine with phenol; some are dressed without staining. A third technique is used, based on the preparation of samples for electronic transmission microscopy. Samples are dehydrated, embedded in an Epon resin, sectioned with an ultramicrotome (thickness of 0.8 µm), stained with methylene blue, and mounted in Eukitt.

Every section was observed under a light microscope (Olympus BX 40). Each cellular type was studied (distribution, number and size of cells, thickness of walls etc.). Photos of the most significant wood features were taken with Pégase Pro software (3.0 version, 2I System, 2000) and with Nikon Coolpix 990 digital camera, installed and adapted on the microscope.

A comparative anatomical study was done with wood reference collections (xylarium, atlases, websites, and

publications). Botanical features were compared first to the native flora, then to the introduced species.

3. Results and discussion

3.1. Anatomical wood description and identification

3.1.1. Wood samples recovered during the BOA 0 cruise (Vanuatu)

3.1.1.1. Big Bay sample CP 2313. It is a hardwood without growth rings (Fig. 1A). Few large-sized vessels are observed in transverse-section (2–3 per mm², up to $285 \times 330 \,\mu\text{m}$ in diameter). Perforation plates are simple, tylosis is present. Intervascular pits are bordered, alternate and measure 6 µm in diameter. Vessel-parenchyma pits are horizontally elongated and larger than the latter. Parenchyma is abundant, in tangential bands of 3 to 8 cells thick. These bands alternate with bands of fibres of the approximately same width. Rays are 4 to 11 seriate (60-140 µm in width), up to 740 µm in height. They are heterocellular with one or two marginal rows of square to upright cells. Sheath cells are present. These features can be related to the genus Ficus L., represented by 750 species in the tropical and warm areas from Indomalaysia to Australia, Africa, and South America [14]. Many species are endemic to the Southwest Pacific Islands of New Caledonia and Vanuatu [2,22,23]. Three species can be considered: Ficus wassa Roxb., F. prolixa Forst. f., F. tinctoria Forst. f. The wood sample is similar to F. prolixa and F. tinctoria, but features of this genus are very close between species and may vary, so the sample is considered as Ficus sp.

3.1.1.2. Epi sample 1 CP 2304. The wood sample is light beige (Fig. 1B). It seems to be a branch with opposite knots. The bark is missing and the core is lost with a narrow canal marking its position, but the sample is well preserved. No traces of colonization by organisms are visible. It is a hardwood, with weakly marked growth rings. Abundant medium-sized vessels are observed in transverse-section (from 18 to 20 per mm²). They are solitary and in multiples of two, three, or four pores. Parenchyma is scarce and paratracheal. Fibres are abundant, with thick walls and usually septate. Rays are not clearly discernible in transverse-section. In tangential longitudinal section, they are arranged irregularly, very tall (up to 3 mm), mostly biseriate, and are constituted of elongated cells (up to 150 µm high, and 11.5–18 µm wide). These kinds of rays are called 'paedomorphic type-I rays' [4]. The anatomical identification of the sample is based on paedomorphic type-I rays. According to Carlquist [4], paedomorphic type-I rays are known in some genera of Asteraceae Martinov (*Baccharis* L., *Brachylaena* R. Br. and *Verbesina* L.) and in some Onagraceae Juss. and Goodeniaceae R. Br. On the one hand, the Asteraceae family must be considered because of a great similarity between our sunken wood and a genus, *Fitchia* Hook. f. [3], known in Polynesia [24]. On the other hand, wood histology of the genus *Fuchsia* L. (Onagraceae), known in Polynesia, is very close [15], and the sample could be attributed to this genus. It appears that the sample is likely to belong to the genus *Fitchia* or *Fuchsia*.

3.1.1.3. Epi sample 2 CP 2304. The wood sample measures 83 cm in length and from 5.3 to 8 cm in diameter (Fig. 1C). It has a clear demarcation between yellowish sapwood and dark brown heartwood. The bark is missing. This sample exhibits abundant depressions and furrows made by limpets, and tunnels bored by bivalves. It is a hardwood, with weakly marked growth rings. Scattered mediumsized vessels are observed in transverse-section, usually 5 per mm². Multiples of two, three, and seven pores were found. Perforation plates are simple. The parenchyma is abundant and predominantly paratracheal, aliform and confluent when the vessels are close. In tangential longitudinal section, parenchyma cells are grouped in vertical rows of 2-6. Crystal chambered cells are present in scattered strands of about 15 cells in the paratracheal parenchyma and among fibres. Rays are uni- or biseriate, homocellular, and 3-36 cells high. Fibres are abundant, with thick walls, and are clearly septate.

These features can be related to the sub-family Mimosoideae Kunth. (Fabaceae Lindley). Among the genera of Mimosoideae encountered in the Vanuatu [2,23], only four of them have septate fibres: *Albizia* Benth., *Inga* Miller, *Leucaena* Benth. and *Serianthes* Benth. [6,15]. Wood histology of the sample is closer to the genera *Leucaena* and *Serianthes* than to the others [1,5,10,13,15], so it is likely to belong to one of them.

3.1.2. Wood samples recovered in the Mediterranean Sea

3.1.2.1. October 2005 sample. It is a ring porous hardwood with large growth rings (Fig. 1D). Big vessels (195 \times 235 μ m in diameter) in early wood are solitary or in radial chains of 2 or 3 pores. They form a continuous ring of one to three layers. Smaller late wood vessels (35 \times 38 μ m in diameter for the smallest ones) are solitary or in small radial chains of 2 to 4 pores, rarely in clusters. They are not disposed with a particular pattern. Perforation plates are simple, tylosis is present. Intervas-

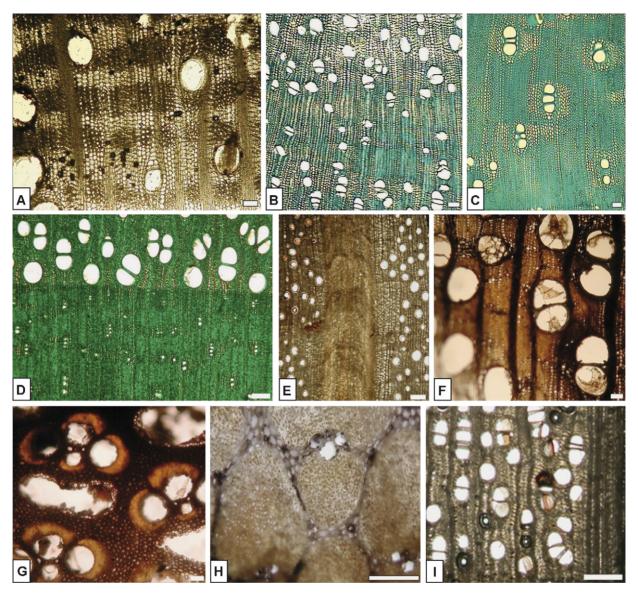


Fig. 1. Transverse sections of identified wood samples. **A**, **B**, **C**: Samples recovered during the BOA 0 cruise. **A**: *Ficus* sp.; **B**: *Fitchia* sp./*Fuchsia* sp.; **C**: *Leucaena* sp./*Serianthes* sp. **D**, **E**: samples recovered in the Mediterranean Sea; **D**: *Fraxinus* sp.; **E**: *Quercus* sp. **F**, **G**, **H**: samples recovered during MUSORSTOM 3; **F**: *Shorea* sp.; **G**: Bambusoideae; **H**: Arecaceae. **I**: *Alnus* sp., recovered during TAIWAN 2002. Scale bars: **A**, **B**, **C**, **F**, **G**, **H**: 100 μm; **D**, **E**, **I**: 200 μm.

Fig. 1 Sections transversales des échantillons identifiés. **A**, **B**, **C**: Spécimens collectés lors de la campagne BOA 0. **A**: *Ficus* sp.; **B**: *Fitchia* sp./*Fuchsia* sp.; **C**: *Leucaena* sp./*Serianthes* sp. **D**, **E**: spécimens récoltés en mer Méditerranée; **D**: *Fraxinus* sp.; **E**: *Quercus* sp. **F**, **G**, **H**: échantillons récoltés lors de MUSORSTOM 3; **F**: *Shorea* sp.; **G**: Bambusoideae; **H**: Arecaceae. **I**: *Alnus* sp., récolté lors de TAIWAN 2002. Barres d'échelle: **A**, **B**, **C**, **F**, **G**, **H**: 100 μm; **D**, **E**, **I**: 200 μm.

cular pits are bordered, alternate and measure 4 μ m in diameter. Walls of vessels are thick (up to 14 μ m). When the parenchyma is paratracheal, sheath cells are disposed in one or two layers, sometimes extending tangentially between two vessels in the late wood. In longitudinal section, cells are of two sizes: 50–70 μ m and 23–33 μ m in length, with thick walls. Rays are homocellular, 1–3

seriate and do not exceed 25 cells in height. In transverse section, the fibres are not disposed in regular rows. No septa or pits are observed. This sample can be related to the genus *Fraxinus* L. Three species are encountered in the Mediterranean area (*Fraxinus angustifolia* Vahl., *F. ornus* L., *F. excelsior* L.), but their anatomical features are very similar [8,18]. The wood sample cannot be rela-

ted with certitude to one of these species, so it will be considered as *Fraxinus* sp.

3.1.2.2. January 2006 sample. In tranverse section, growth rings are just marked by rectangular fibres with thicker walls (Fig. 1E). The distribution of the vessels is radial or dendritic (flame-like), sometimes nearly diffuse. The bigger vessel diameter is up to $117 \times 140 \,\mu m$. Perforation plates are simple, intervascular pits are alternate. Parenchyma cells are diffuse-in aggregates or in tangential undulating lines of one cell in thickness, separated by 1 to 10 fibre layers. Rays are uniseriate (up to 23 cells high) or multiseriate. These ones are very large (up to 670 μm), measuring several millimetres in height, often dissected by fibres in several units at the ends and margins (tangential longitudinal section). Cells are often crystalliferous. Tracheid and libriform fibres are observed. This sample can be related to the genus Quercus L. It has the structure of an evergreen oak, and probably belongs to the species Quercus ilex L. or Q. coccifera L., Q. ilex being more frequent than Q. coccifera in the Mediterranean area [17].

3.1.3. Wood samples recovered during MUSORSTOM 3 cruise (Philippines)

3.1.3.1. Sample 1. The wood is bored with many small tunnels (bivalves) (Fig. 1F). It is a hardwood without growth rings. Vessels are solitary (up to 330 μm in diameter) and radially grouped by two. Tylosis and vestured pits are present. The parenchyma is paratracheal and in sinuous tangential bands (1–4 cells thick). Rays are heterocellular, up to 4 seriate and possibly exceeding 1 mm in height. Small axial canals in tangential lines are sometimes observed. This sample can be related to the genus *Shorea* Roxb. ex Gaertner f. (Dipterocarpaceae Blume) [9]. This genus regroups about 350 species from Sri Lanka to southern China, the Moluccas and Lesser Sunda Islands. It is well represented in the Philippine Islands [14].

3.1.3.2. Sample 2. The sample exhibits small tunnels bored by bivalves (Fig. 1G). Culms with central cavity, primary fibro-vascular bundles are observed. This structure is characteristic of a Bambusoideae Luerss.

3.1.3.3. Sample 3. Very dense primary fibro-vascular bundles are observed (Fig. 1H). This structure is characteristic of an Arecaceae Schultz-Schultzenst.

3.1.3.4. Wood sample recovered during TAIWAN 2002 cruise. It is a diffuse porous hardwood (Fig. 1I). Vessels are solitary and radially grouped. Perforation plates

are scalariform with numerous bars. Parenchyma is diffuse and diffuse-in aggregates. Rays are uniseriate and aggregated in 'false' wide rays.

This sample can be related to the genus *Alnus* Miller (Betulaceae Gray), probably to *A. formosana* (Burkill) Makino, endemic from Taiwan [10].

4. Conclusion

Despite long underwater immersion, identification of sunken woods is still possible. Wood samples had a well-preserved structure, which allowed a classical preparation (sectioning with a razor blade or a sliding microtome). Samples were accurately determined. These identifications show a diversity of taxa for each sampling area (except for the Taiwan area, for which only one wood sample was studied). Samples are seemingly native to local floras, but the *FitchialFuchsia* sample (recovered off the coast of the Vanuatu archipelago) may have been an introduced species.

The successful identification of sunken wood allows further studies on this particular ecosystem. Botanical diversity of sunken wood linked with faunal inventory will be studied to identify specific associations between colonising organisms and substrates. More samples will be observed, recovered during the BOA cruises around Vanuatu. *In situ* experiments are in progress, with a view to studying degradation and colonisation processes of different known wood species placed at the same depth, in the same conditions and for the same duration.

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