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Presence of *Camptotheca* in the Red River Delta (North Vietnam) during the Holocene Revealed by Palynological Studies

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Authors' Contributions

This work was carried out in collaboration between all authors. Author NTD performed the pollen analyses and interpreted the dataset. Author PDK performed additional literature research and wrote the text of this paper. Author HJ conceived and supervised the research project, cooperated in the interpretation of the dataset and edited the text of the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Evaluation of the presence of the important medicinal tree genus *Camptotheca* in the Red River Delta (northern Vietnam) during the Holocene.
Place and Duration of Study: Institute of Botany and Landscape Ecology (Greifswald University), between March 2003 and May 2006.

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Methodology: Pollen analyses were performed on various sediment cores from the Red River Delta in order to reconstruct vegetation history and landscape development during the Holocene. Radiocarbon dates of selected levels provide a chronologic framework for various vegetation phases.
Results: *Camptotheca* occurred with certainty in the Red River Delta, i.e. outside its current distribution area, around 6500 and somewhat after 6150-5500 cal yr BP. It was probably present during the complete 7400-5300 and 1400-250 cal yr BP time-slices. Presence during other time slices is possible, but could not be confirmed.
Conclusion: *Camptotheca* ecologically represented a specific phase in riparian forest development, where it followed on *Carya*, *Pterocarya* and *Salix* during stages when the forest was relatively open. This study is a first step in understanding the past natural ecology of *Camptotheca*, which may provide information useful for management of *Camptotheca* plantations for medicinal purposes.

Keywords: Camptotheca; happy tree; palaeoecology; palynology; Red River Delta; riparian forest; vegetation; Vietnam.

1. INTRODUCTION

The genus *Camptotheca*, including the two tree species *C. acuminata* (Xi Shu, Chinese Happytree) and *C. lowreyana* [1], is of great medicinal importance because it provides effective medicines against various forms of cancer and Human Immunodeficiency Virus (HIV-1) [2-9]. Some taxonomical studies include the genus in the Cornaceae or Cornaceae s.l. family [10-12], whereas others place it - together with the genus *Nyssa* and often also with *Davidia* - in a separate Nyssaceae family [1,2,13,14]. *Camptotheca* is considered to be a relict genus, with more members and a wider distribution during the Tertiary [3].

Currently, the genus *Camptotheca* is only native in central and southern China (including the province Yunnan that borders on Vietnam), and in Tibet [1,3,12,15]. Presence is also expected for Burma and northern Thailand [2]. The taxon has been introduced in the USA, Japan, South Korea, and the United Kingdom [2,3,6]. Although its presence in Vietnam has been suspected, actual specimens have not been found yet [16].

Camptotheca acuminata occurs in regions with high temperatures, high precipitation and high relative humidity [2], normally below 1000-1500 m asl. [1,3,12,17] although occasional occurrences up to 2400 m asl. have been observed [2,3]. It prefers moist and fertile sites on deep, well-drained, friable clay soils [2,3], predominantly along forest margins and streams, and on slopes, where it frequently grows in thickets [3,17] reaching heights up to 20-30 m [2,4,17]. The species is drought- and shade-intolerant [3], although it has also been reported to occur under conditions with only little direct sun light [2].

Although experimental planting studies revealed much detail on the growing requirements of *Camptotheca acuminata* especially in relation to optimal production of its agent Camptothecin (CPT) [6,8,18-21], little is known about the Holocene history of the genus and its ecological behaviour under natural conditions.

During recent research into the Holocene development of the northern Vietnamese Red River Delta, several palynological samples were found to have high values of pollen grains attributable to *Camptotheca* [22,23]. The present paper evaluates these finds and discusses the past distribution and ecology of the genus.

2. MATERIALS AND METHODS

2.1 Study Area

The Red River (Song Hong) crosses northern Vietnam in southeastern direction in an almost straight line from the mountains at the Chinese border to the Gulf of Tonkin of the South China Sea (Fig. 1). The river has developed a large delta towards the coast, with up to 80 m thick deposits of Weichselian Late glacial and Holocene age [22,24-30].

The higher areas in northern Vietnam (above 700 m asl) are covered by a diverse forest vegetation, whereas the tropical evergreen broad-leaved forests of intermediate elevations (100-700 m asl) have partly been replaced by agricultural fields and secondary plant communities [31,32]. The fluvial lowland (below

100 m asl) is expected to have consisted mainly of wetland forests [32] which now have been completely replaced by agricultural fields, with rice being the most important crop [32-34]. Extensive mangroves in the river mouth area [35-37] have greatly been reduced in area by human activities [32].

2.2 Research Methods

Several long-sequence cores were taken along the Red River and its tributaries (Fig. 1). Sample preparation included treatment with HCl and KOH, sieving (meshes 120 µm), treatment with HF, acetolysis (7 min), and mounting in silicone oil [38]. Counting was with a Zeiss binocular microscope with a magnification of 400 times; larger magnifications were used for the identification of problematic palynomorphs.

Pollen and spores were identified with and named after various pollen morphological studies [39-43]. Additionally, a pollen reference collection was used. Observed palynomorphological types are in the text of this paper displayed in SMALL CAPITALS in order to differentiate them clearly from inferred plant taxa [44,45].

As inclusion of pollen types from (extra)local vegetation elements in the pollen sum may substantially distort representation of regional pollen values [46], all pollen values were calculated relative to a sum of types that are produced by plant taxa currently growing above 100 m asl, i.e. outside the delta. In order to facilitate description, interpretation and discussion of the pollen diagrams, the pollen types were ordered stratigraphically in different ecological groups [47] on the basis of the current ecological requirements of the inferred plant taxa [16,48-51]. The pollen diagrams were divided by visual inspection into site pollen zones [52] that are a combination of informal acme zones and informal interval zones [53].

Photos of CAMPTOTHECA pollen from core HN were taken with different focus-levels and subsequently stacked to one image using the Helicon Focus software.

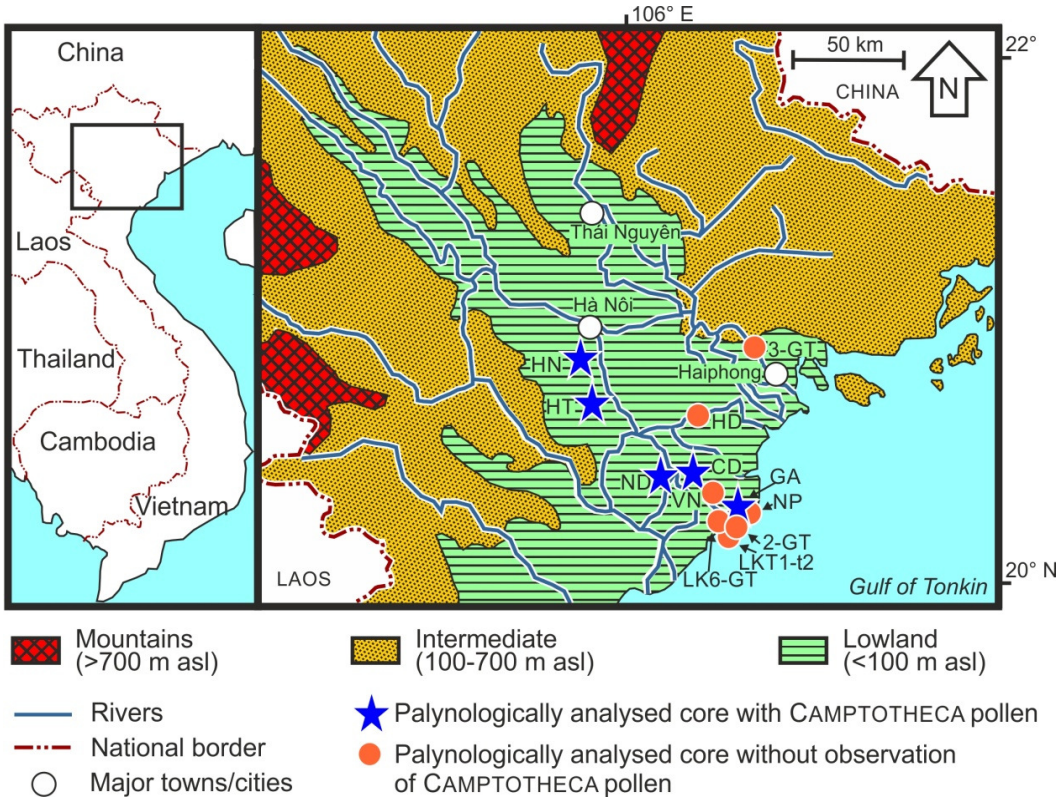

Fig. 1. Map of the Red River delta (N Vietnam) and sites referred to in this paper
CD (Chuongduong), HN (Hanoi) and HT (Hatay): [22,23,26] and this paper; HD (Haiduong) and NP (Namphu) [22,26]; LK6-GT, LKT1-t2, 2-GT and 3-GT [27]; ND-1 [28]; GA and VN [29,34]

Three radiocarbon dates of wood and peat and one of mollusc shells of relevant depths (Table 1) were calibrated to calendar years BP with the CALIB 6.1.0 computer program [54] using the Intcal09 calibration set for the samples of wood and peat and the Marine09 calibration set for the sample from shells [55]. ΔR and ΔR-error values were retrieved from http://www.calib.qub.ac.uk/marine/, which provides three completely different ΔR and ΔR-error values for the Xisha islands (16.7000 N, 112.3000 W), the location in the dataset closest to the Red River Delta (distance ca. 700 km). Differences between the three calibrations performed for the mollusc sample (Table 1) were, however, so small that they do not impair chronological interpretation.

3. POLLEN OF *Camptotheca*

Camptotheca is a polygamous taxon with both male and hermaphrodite flowers [3,17,56]. Usually, 30-60 flowers form a head [3,57], with each flower - that contains 10 anthers [56] - producing around 7000-8000 pollen grains [58]. Also the hermaphrodite flowers produce pollen [56]. Flowering is reported for May to July [10] or for July and early August [2]. Pollen is dispersed by various insects [3,57,58].

Morphological descriptions of *Camptotheca* pollen in pollen morphological literature are not completely consistent. The following summary uses terms standardised after the "Glossary of pollen and spore terminology" [59].

The outline of the pollen grain has been described as triangular [60], oblate-triangular to almost hexagonal [61], oblate-spheroidial [41,62], or slightly concave triangular [63].

Whereas most studies mention a scabrate pollen wall structure, one study describes the grain as verrucate to regulate [61], and one as baculate [62].

The pollen is tricolporate; only one study describes the grain as tricolpate but with a 'distinct germinal aperture' within the colpi [60]. The pores are large and protruding, and according to three studies [41,62,63], the pori have costae. The pori are described as ellipsoidal [61], lalongate [64], transverse [41], round [62] or elliptic to roundish-triangular [63].

Large discrepancies in description exist with respect to the colpi, which are called long [60], of medium length [61], or short [63]. Whereas some authors state that the colpi have costae [63, 64], others mention a thinning of the exine along the colpi [41,61]. Another study mentions both a thickening and a thinning along the colpi [62]. The colpi are furthermore described as broad and tapering with nearly smooth membranes [60], boat-shaped with distinct margins [61], and as not very distinct [64].

Table 1. Radiocarbon dates of cores HN, HT and CD

Core	Sample	Depth (m)	Lab. nr.	Dated material	Conventional age (^{14}C-yr BP)	Calibrated age (cal yr BP)
HN	HN-07	7.25	KIA27458	Wood, base residue	5712 ± 36	Calset Intcal09 6570-6410 6630-6590
HT	HT-02	5.31	KIA27453	Wood, base residue	5040 ± 120	Calset Intcal09 5510-5490 6020-5580 6060 6110-6080 6170-6160
HT	HT-10	16.05	KIA27454	Peat/wood, base residues	7507 ± 32	Calset Intcal09 8260-8210 8390-8290
CD	TB3-11	14.30	KIA27450	Carbonate from shells	5985 ± 37	Calset Marine09 ΔR=11; ΔRerror=40: 6510-6270 ΔR=-10; ΔRerror=50: 6560-6280 ΔR=-73; ΔRerror=60: 6480-6190

Pollen grain sizes are (polar axis x equatorial axis) 27-31 x 40-46 µm (P/E ratio thus c. 0.67-0.68) (mounted in glycerine) [63], 29-38 x 39-54 µm (P/E ratio c. 0.70-0.74) (mounted in glycerine jelly) [61], 29 x 39 µm (P/E ratio 0.74) (in glycerine jelly) [64], 27-36 × 39-48 µm (P/E ratio 0.69-0.75) (in glycerine) [41], 30.1 x 37.3 µm (P/E ratio 0.81) (mounting medium unknown) [62], and 33.2 x 41.1-µm (P/E ratio 0.81) (mounted in lactic acid) [60].

Pollen grains of *Nyssa* are very similar, but have a larger size than those of *Camptotheca* [41,60,61,63,64]. The pollen of both genera can be differentiated by the distinct oblate shape and hexagonal outline of *Camptotheca* pollen [61]. The paracolpus (i.e. wall thinning) of *Camptotheca* pollen is more closely connected to the colpus than that of *Nyssa* [62]. Somewhat similar but nevertheless distinguishable pollen types are mentioned for *Mastixia* [60], some Cornaceae (without specification) [60,64], and *Cynoxylon*, *Cornus* and *Chamaepericlymenum* [62].

The pollen identified by us as CAMPTOTHECA (Fig. 2) is tricolporate and has a scabrate wall structure. In polar view, the grain is triangular and has straight to slightly concave walls. In equatorial view, the grain is distinctly oblate. The colpi are broad, distinctly costate and of moderate length. The pores are markedly lalongate (and are perhaps more accurately described as transverse endocolpi). These characteristics are largely compatible with the morphological descriptions summarised above. The grains identified by us have a polar axis of c. 22 µm and an equatorial axis of around 30 µm (P/E ratio c. 0.73) and are thus somewhat smaller than the grains described in the literature. This observation is in good agreement with the fact that pollen grains mounted in silicone oil are smaller than those mounted in glycerine or glycerine jelly [65,66].

4. RESULTS AND DISCUSSION

In the pollen diagram of core HN (Fig. 3), zone HN-B shows high values of pollen types attributable to typical mangrove and back-mangrove taxa such as *Kandelia*, *Rhizophora*, *Luminitzera*, *Acrostichum*, *Bruguiera*, and *Scyphiphoria*. This demonstrates that during the time-span covered by this zone the site was located within the mangrove zone, i.e. the sea extended far land inward compared to its current coastline. Pollen types of riparian trees show a conspicuous succession of CARYA (zone HN-B), PTEROCARYA (HN-B/HN-C) and SALIX (HN-C), above which a large peak of CAMPTOTHECA pollen occurs (zone HN-D) that dates to 6630-6410 cal yr BP. The type also occurs with low values in the samples adjacent to the boundary between pollen zones HN-A and HN-B. Probably stream-bank forests formed a separate habitat that existed within the mangrove environment and developed further after the mangroves had disappeared from the site.

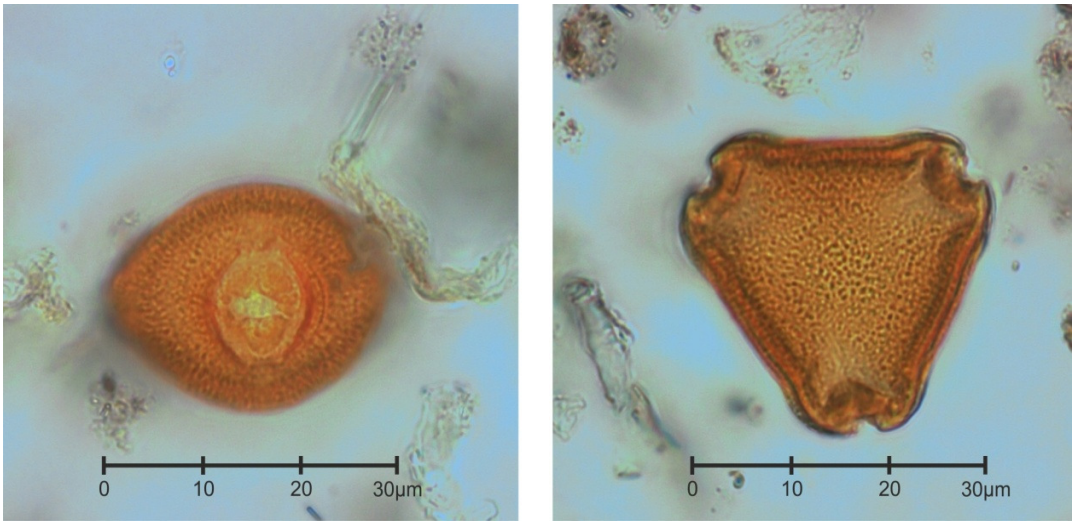

Fig. 2. Pollen identified as *Camptotheca* from core HN

Equatorial view (left) and polar view (right). Various photos taken with different focus-levels were stacked using the Helicon Focus software

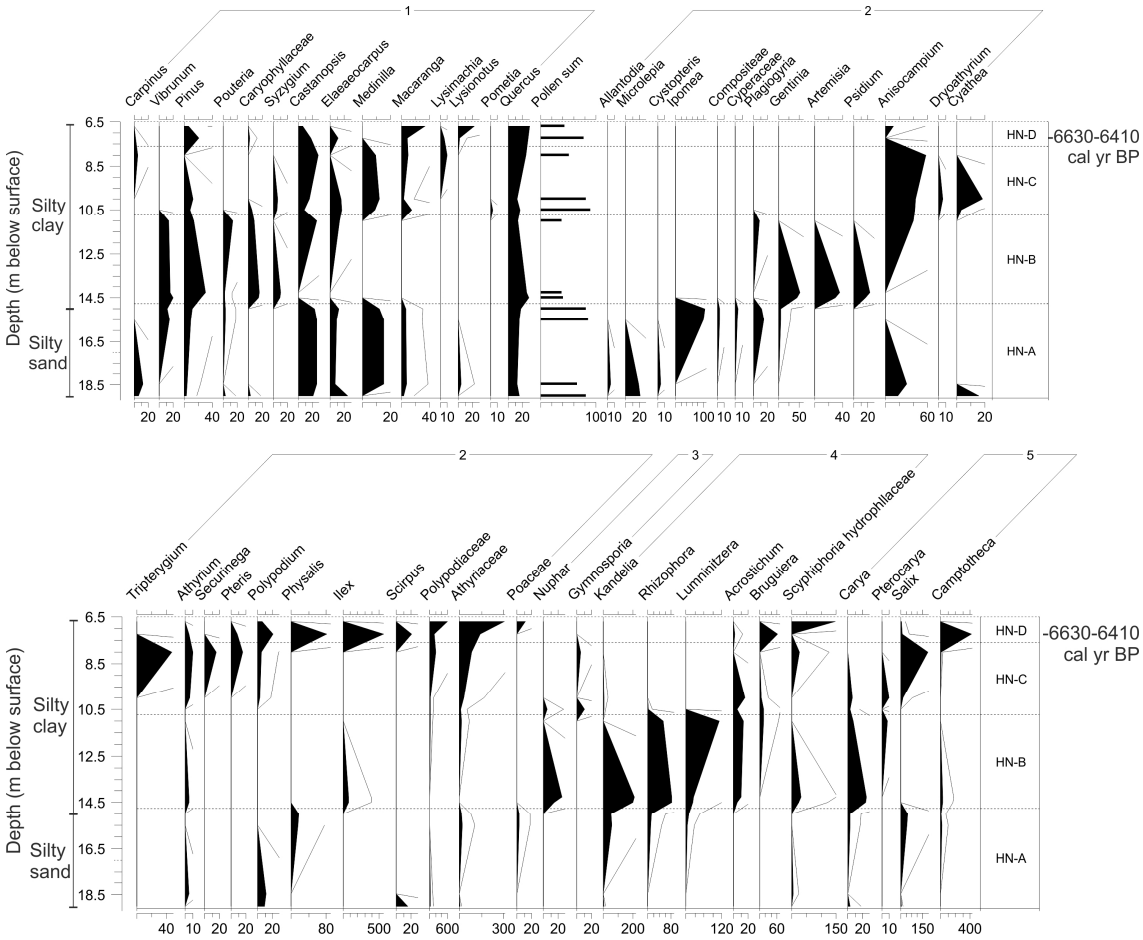

Fig. 3. Pollen diagram of core Hanoi (HN) – selected types and selected depth levels

Pollen type groups: 1. Types of plant taxa growing above 100 m asl; 2: types of taxa covering broad ecological ranges; 3: types of open water taxa; 4: types of (back-) mangrove taxa; 5: types of riparian taxa

Pollen diagram HT (Fig. 4) does not show a typical mangrove phase within the selected depth trajectory. CAMPTOTHECA pollen occurs only in the top sample, which clearly dates younger than 6170-5490 cal yr BP. In zones HT-C and HT-D, again a succession of peaks occurs of subsequently CARYA, PTEROCARYA, SALIX and CAMPTOTHECA pollen, although this succession is less conspicuous.

Diagram CD (Fig. 5) shows over the entire selected depth trajectory high values of pollen types produced by typical (back-) mangrove taxa (including *Cyperus*, *Kandelia*, *Acrostichum*, *Bruguiera* and *Rhizophora*). CAMPTOTHECA pollen occurs in zone CD-C in low values (up to 10%) that clearly date from before ca. 6560-6190 BP. A succession is discernible of SALIX, PTEROCARYA, CAMPTOTHECA, and again PTEROCARYA pollen.

Pollen attributed to *Camptotheca* was also found in the cores ND-1 [28] and GA [29] (cf. Fig. 1). Whereas these papers do not specify the amount and the time-slices in which CAMPTOTHECA pollen was found, Li Zhen (pers. comm. May 2012) informed us that various samples with only few CAMPTOTHECA grains date between ca. 7400-5300 cal yr. BP (core ND-1), and between ca. 1400-250 cal yr BP (core GA). CAMPTOTHECA pollen was not observed in cores HD and NP [22, 26], VN [29] and 2-GT, 3-GT, LK&-GT and LKT1-t2 [27] (cf. Fig. 1).

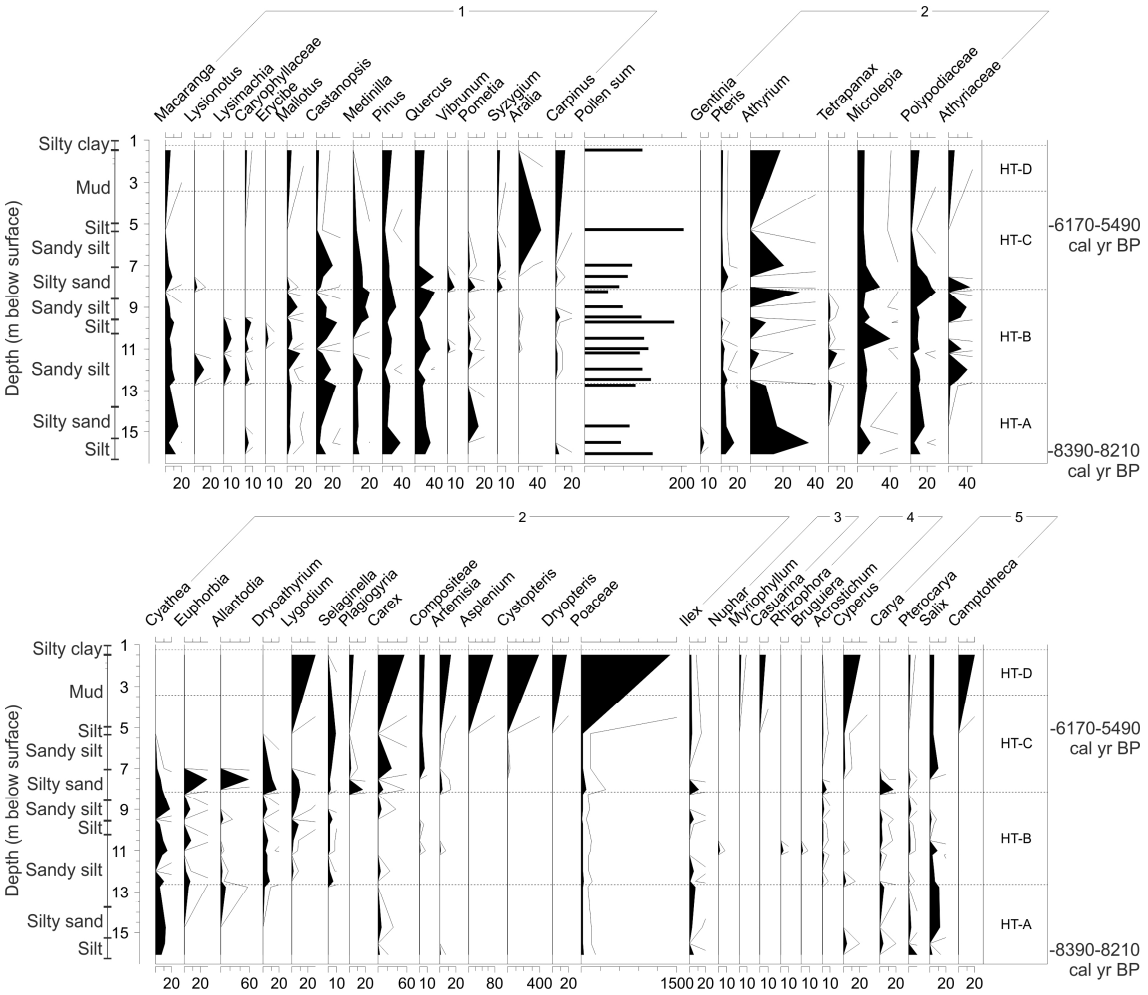

Fig. 4. Pollen diagram of core Hatay (HT) – selected types and selected depth levels

Pollen type groups: 1. Types of plant taxa growing above 100 m asl; 2: types of taxa covering broad ecological ranges; 3: types of open water taxa; 4: types of (back-) mangrove taxa; 5: types of riparian taxa.

Since pollen attributable to CAMPTOTHECA does not occur continuously but in a small number of samples only, pollen dispersal apparently was limited, as one may expect from insect-pollinated plants. The low values in cores GA and ND-1 may originate from regional or extraregional pollen sources [46,67], but the high values of CAMPTOTHECA pollen in cores HN and HT unambiguously show presence of *Camptotheca* trees at or directly around the core locations. Whether the values in core CD represent regional pollen deposition only or already (extra) local deposition of the taxon is difficult to say without detailed studies on pollen dispersal of *Camptotheca*. The latter possibility seems likely compared to the absence of CAMPTOTHECA pollen in other palynologically analysed sites, but a possible overinterpretation should be avoided.

The succession of various pollen types of riparian trees indicates that the occurrence of *Camptotheca* trees represents a specific phase in the development of stream-bank forest, where it followed on members of the genera *Carya*, *Pterocarya* and *Salix*. Since information on the ecology of *Camptotheca* and the vegetation types in which it occurs is limited (cf. text-section 1), it is hazardous to speculate greatly on the actual habitats of the genus. However, since *Camptotheca* is shade-intolerant, it is safe to conclude that sufficient open spots must have been present in the wetland forests and that populations diminished when the forests closed-up.

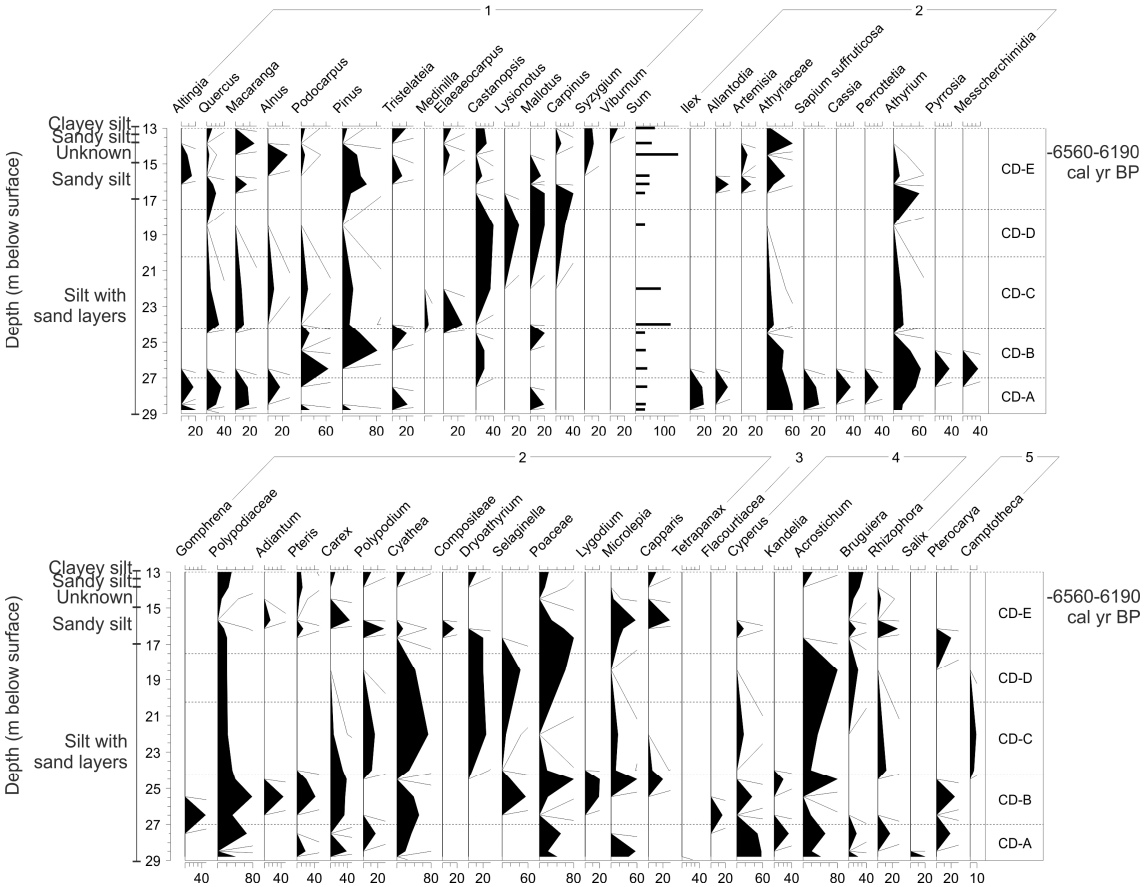

Fig. 5. Pollen diagram of cores Chuongduong (CD) – selected types and selected depth levels

Pollen type groups: 1. Types of plant taxa growing above 100 m asl; 2: types of taxa covering broad ecological ranges; 3: types of open water taxa; 4: types of (back-) mangrove taxa; 5: types of riparian taxa

It is not yet possible to estimate accurately how widespread *Camptotheca* may have been in the Red River Delta, but since its pollen was observed at various sites within the delta the genus must have been common, be it localized and associated with specific succession stages. Low pollen production and poor pollen dispersal (as discussed above) may explain why the pollen type was not found in all cores.

The data of cores HN and HT demonstrate positively that specimens were present in the direct vicinity of these sites around 6500 and somewhat after 6150-5500 cal yr BP respectively. When the values of CAMPTOTHECA in the pollen diagrams from cores CD, ND-1 and GA reflect presence of *Camptotheca* trees somewhere in the region, presence of the genus in the delta is likely for the time slices 7400-5300 cal yr BP and 1400-250 cal yr BP. It is very well possible that the taxon also occurred during other time-slices, but hard conclusions cannot be drawn from the data currently available.

The palaeoecological record does not provide much indication on why *Camptotheca* is currently absent from the delta, but its occurrence up to a few centuries ago makes it tempting to assume anthropogenic impact as a cause, e.g. cultivated areas gradually expanding at the expense of natural wetlands forests.

5. CONCLUDING REMARKS

Camptotheca was in the past no rare element in the north-Vietnamese Red River delta, i.e. outside the range of its current natural distribution area. The taxon very likely occurred in specific phases of riparian forest succession when the tree vegetation was rather open: *Camptotheca* specimens may have disappeared later as consequence of the development of denser forests. Future palynological research in

the Red River Delta may reveal other localities and time-slices of past occurrences of *Camptotheca*, which will provide more detailed knowledge on the abundance, distribution, development and decline of *Camptotheca* populations during the Holocene. This knowledge will result in a better understanding of the ecology and the natural behaviour of this medicinal important tree genus and could be useful to optimize cultivation of the taxon for medicinal purposes.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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