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Chemotaxonomy of *Taxus*

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Abstract

A chemotaxonomical study of the genus *Taxus* was undertaken, based on the concentration of six neutral taxanes in its needles. A total of 750 different samples from 10 different *Taxus* species or hybrids were analysed. The chemical data were processed by Cluster and Principal Component Analysis. The results of the chemical analyses allowed the distinction of a North American and a Eurasian group of species. Cultivars belonging to the closely related species *T. baccata*, *T. cuspidata* and *T. x media* could be divided in five major groups based on morphological data. However, a classification of these same cultivars based on the chemical data showed no correlation between taxane content and morphology. The chemical dataset showed some separation of the species *T. baccata*, *T. cuspidata* and *T. x media*, but did not indicate an intermediate position for *T. x media*, but rather a heterosis effect for the hybrids. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Taxus L. is a problematic genus, not only because the distinction between the species is difficult, but also because of the doubtful circumscription of its family, the Taxaceae and even its order, the Taxales. *Taxus* species are used as the source of paclitaxel (Taxol®, Yewtaxan®), an antitumor drug which was first isolated from the bark of the Pacific yew (*Taxus brevifolia* Nutt.) (Wani, Taylor, Wall, Coggon & McPhail, 1971). Paclitaxel belongs to a group of secondary metabolites collectively indicated with the name taxanes. Taxanes are found in most yews, but the content is very variable. To find suitable plants for the production of paclitaxel, approximately 750 different yew samples were screened for their taxane content (van Rozendaal, Lelyveld & van Beek, 1999). The size of this study made it possible to carry out a chemotaxonomical study of the genus *Taxus*, based on the occurrence of the taxanes analysed. The outcome of the chemotaxonomical study was compared with a morphological classification of *Taxus*. Additionally, some species from other genera from *Taxus* within the Taxaceae were screened for their taxane content. Taxanes proved to occur exclusively in the genus *Taxus*.

2. Results and discussion

From a botanical point of view the Taxaceae have always been a point of discussion. Sometimes the family was placed in a separate order, the Taxales (Florin, 1948) and sometimes fused with the Podocarpaceae and the Cephalotaxaceae (Buchholz, 1934). *Cephalotaxus* was placed by some authors into the Taxaceae (Shani, 1920; Koidzumi, 1942) but most authors placed *Cephalotaxus* in a separate family. The Podocarpaceae are probably related to the Taxaceae through *Phyllocladus* and *Austrotaxus* (Chaw, 1996).

The position of *Taxus* within the Taxaceae is not

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Table 1
Distribution of the different *Taxus* species

Taxus species	Distribution
T. baccata	Europe and Asia
T. brevifolia	northwest Pacific
T. canadensis	Canada
T. celebica	Asia
T. cuspidata	Japan
T. floridana	northwest Florida
T. globosa	Mexico/El Salvador
T. wallichiana	Himalayas

problematic for most authors, it is the type genus of the family. Most authors consider *Taxus* to contain about eight species: *Taxus baccata* L., *Taxus cuspidata* Sieb. and Zucc., *Taxus canadensis* Marshall, *Taxus brevifolia* Nutt., *Taxus floridana* Chapm., *Taxus globosa* Schltdl., *Taxus wallichiana* Zucc. and *Taxus chinensis* (Pilger) Rehder. Furthermore, two hybrids exist. *Taxus x media* Rehder is a cross between *T. baccata* and *T. cuspidata* and *Taxus x hunnewelliana* Rehder is a cross between *T. cuspidata* and *T. canadensis*. Other authors, such as Pilger, however, think that *Taxus* consists of only one species, *T. baccata*, with local variations in habit and leaf form (Hartzell, 1991). Table 1 gives the distribution of the different *Taxus* species.

If the genus is not considered as one species, it is divided into several species on the basis of morphological criteria and the occurrence of most species in limited areas of distribution. Krüssmann tried to give essential characteristics by which a species could be distinguished, but all these characteristics are debatable and depend on age and stage of development of the plant (Krüssmann, 1972). Most species are morphologically highly variable and plants with a similar phenotype can be found within different species. In such cases, it appears that species can only be distinguished on the basis of their origin. It would surely be possible to go back to Pilger and consider all 'species' as varieties of *T. baccata*, but our knowledge of *Taxus* would not increase through such a decision.

Most yews are found in the northern hemisphere.

Table 2
Characteristics of the morphological classification

(1) Sex
(2) Colour of needles
(3) Needles unpatterned upside
(4) Length of needles
(5) Habit
(6) Weeping
(7) Growth rate
(8) Dwarf growth
(9) Density of the needles
(10) Needles straight or curving
(11) Colour changes after the first growing season
(12) Colour of the aril
(13) Branches contorted
(14) Height
(15) Needles width
(16) Needle arrangement
(17) Density of branches
(18) Density of branchlets

Only the Asian yews cross the equator in Sumatra. The Eurasian species are the only ones that have overlapping areas of distribution, the others are geographically strictly separated.

The results of the phenetic analyses of the chemical data for the species are presented in Figs. 1 and 2. Both in the dendrogram and the plot of the first two principal components *T. brevifolia* appears rather isolated, while the rest of the species can be divided in two major groups:

1. a North-American group: *T. canadensis*, *T. floridana* and *T. globosa*.
2. an Eurasian group: *T. baccata*, *T. celebica*, *T. cuspidata*, *T. x hunnewelliana* and *T. x media*.

These groups seem more or less natural because the Eurasian group has no real borders between the species, while it is clearly separated from the North-American group by the Atlantic and Pacific oceans. *T. brevifolia* was the only species that contained a very high content of brevifoliol and this accounts for its isolated position. When brevifoliol was not taken into account *T. brevifolia* was placed in the North-

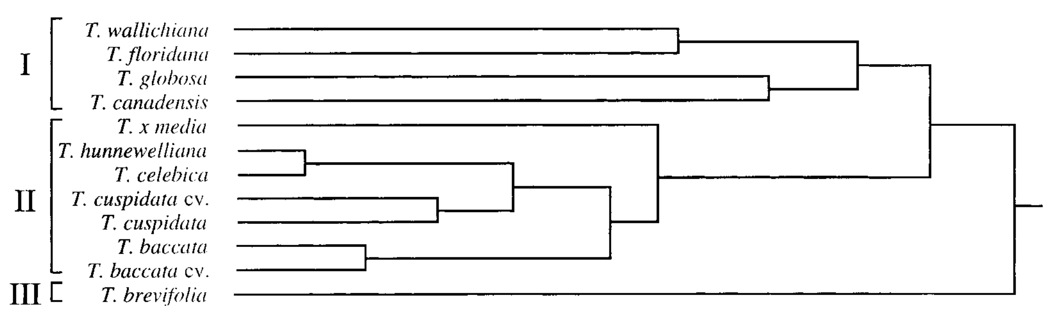

Fig. 1. Dendrogram at the species level based on chemical data.

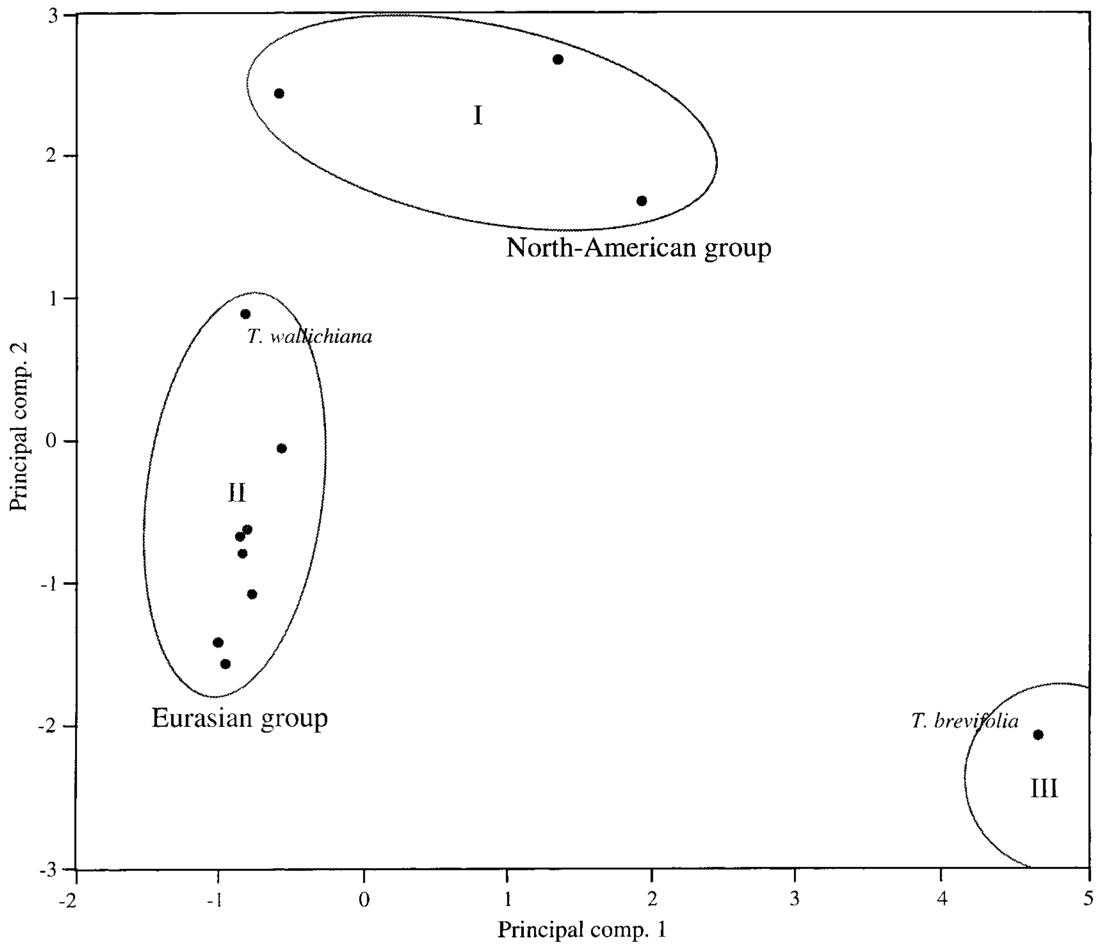

Fig. 2. Principal component analysis at the species level based on chemical data.

American group. The position of *T. wallichiana* (occurring in the Himalayas from Pakistan to Tibet) is unclear. In the dendrogram this species clusters with the American species, while in the principal component analysis (PCA) it is closer to the Eurasian group. The correlation in geographical distribution opposes the opinion of Pilger that all species can be reduced to variants of *T. baccata*.

In the morphological part of this study eighteen different characteristics were used (Table 2). A dendrogram based on the morphological data of the cultivars of *T. baccata*, *T. cuspidata* and *T. x media* is presented in Fig. 3. Five major groups can be distinguished. The relationships among the groups are uncertain because the distances between the groups are very small. The division of the cultivars in these groups, however, is clear. The five groups, which were each named after an important feature are:

1. Aurea group: variegated *T. baccata* cultivars.
2. Media group: cultivars ascribed to *T. x media*.
3. Wild group: wild trees and cultivars similar to wild trees of *T. baccata* and *T. cuspidata*.
4. Nana group: mainly dwarf plants and other deviating cultivars.
5. Fastigiata group: cultivars belonging to *T. baccata*, easily recognisable by their peculiar leaf arrangement, positioned radially around the branchlets.

These groups can be seen as formal cultivar-groups in the sense of the International Code of Nomenclature for Cultivated Plants (ICNCP) to accommodate cultivated material in *Taxus* (Trehane, 1995).

A dendrogram of the same cultivars, based on the chemical data, is presented in Fig. 4. There appears to be no correlation between the groups based on morphological and those based on chemical data. Because yew cultivars were not developed to produce chemical compounds, no selection for these traits has taken place and chemical composition varies widely within and among the morphological groups.

A PCA based on chemical characters for the studied cultivars, coded by species (*T. baccata*, *T. cuspidata*, *T. x media*) is shown in Fig. 5. The representatives of the three species form partly overlapping groups, with the *T. cuspidata* cultivars more or less in between those of

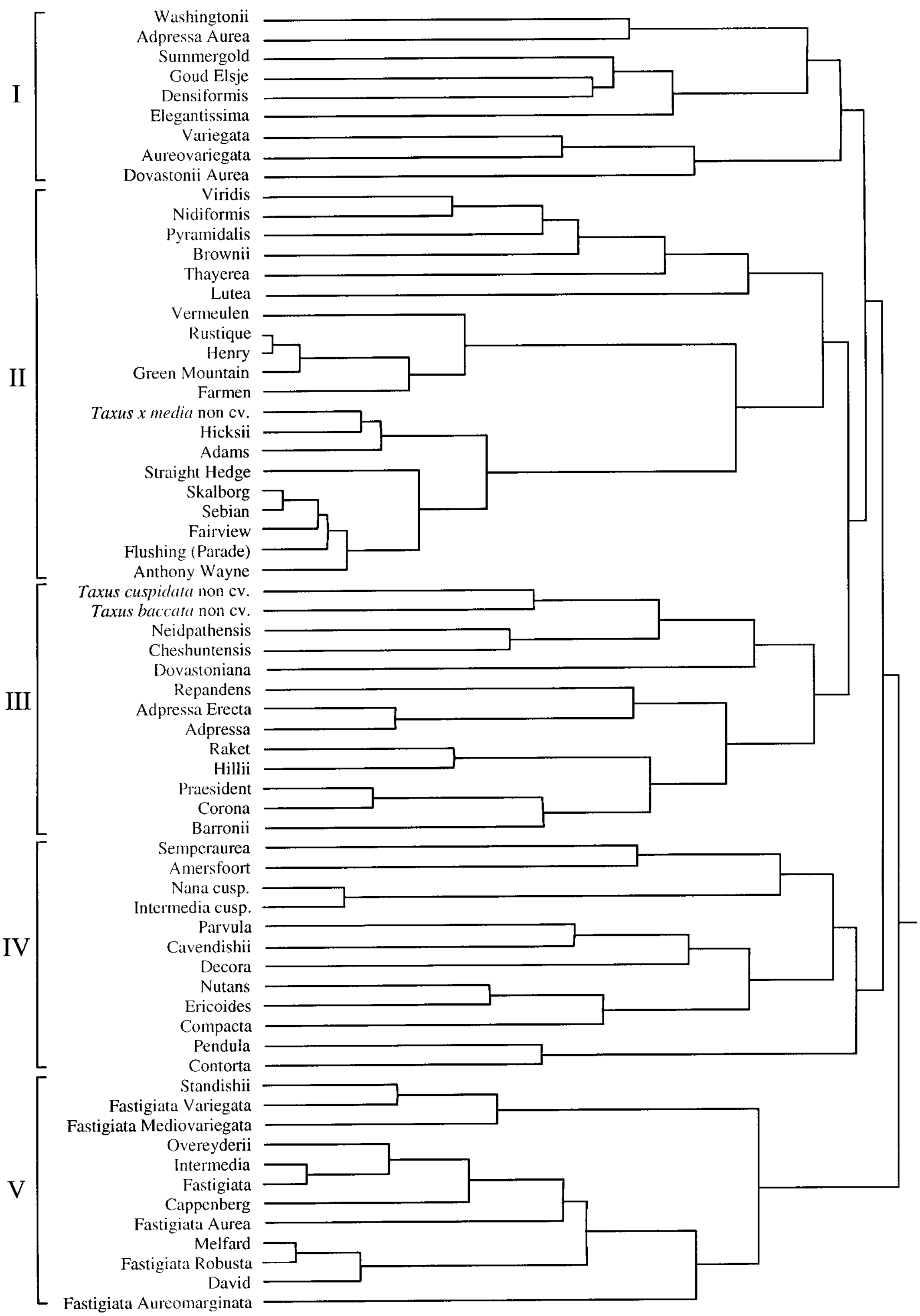

Fig. 3. Dendrogram based on morphological data for the cultivars of *T. baccata*, *T. cuspidata* and *T. x media*.

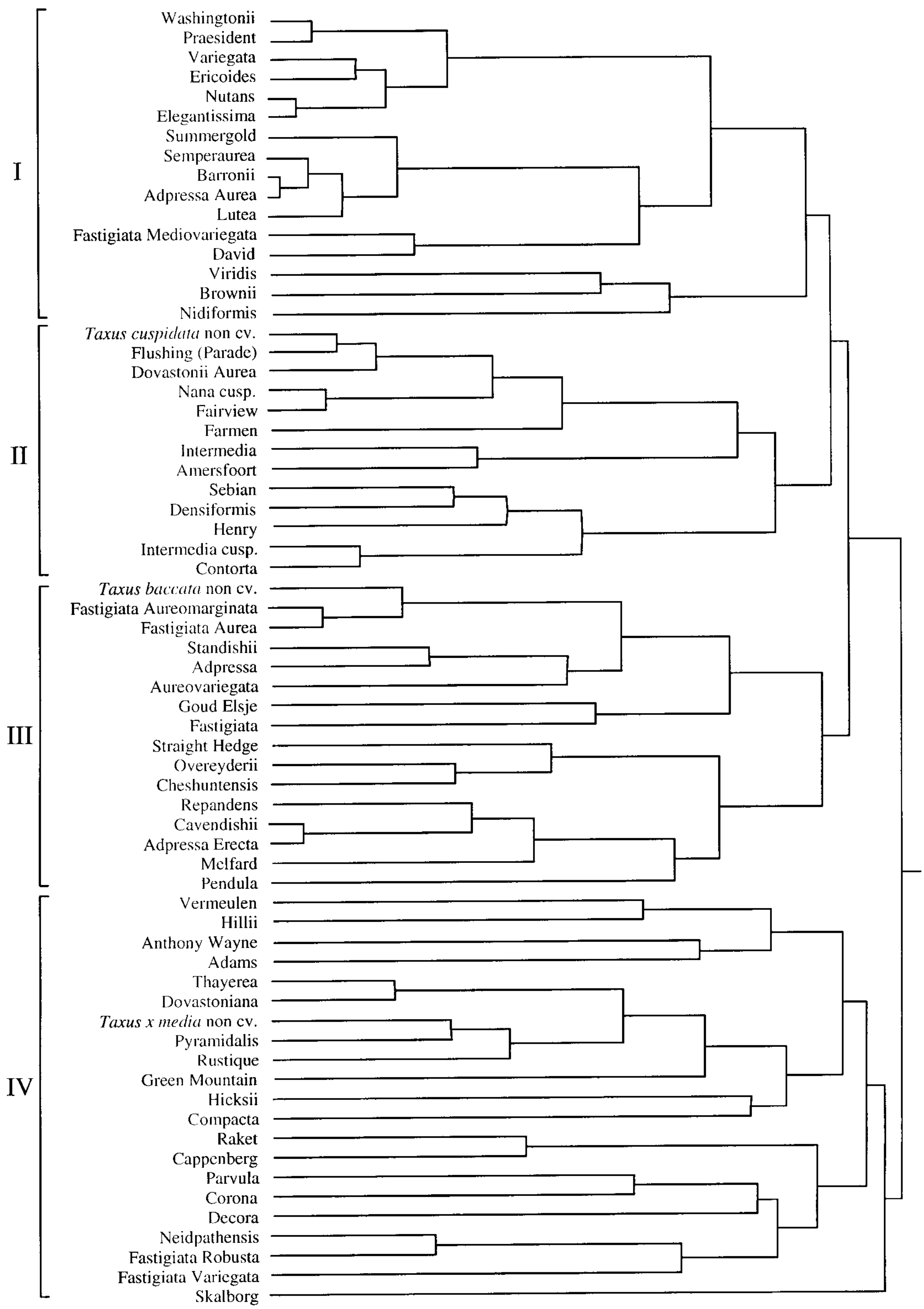

Fig. 4. Dendrogram based on chemical data for the cultivars of *T. baccata*, *T. cuspidata* and *T. x media*.

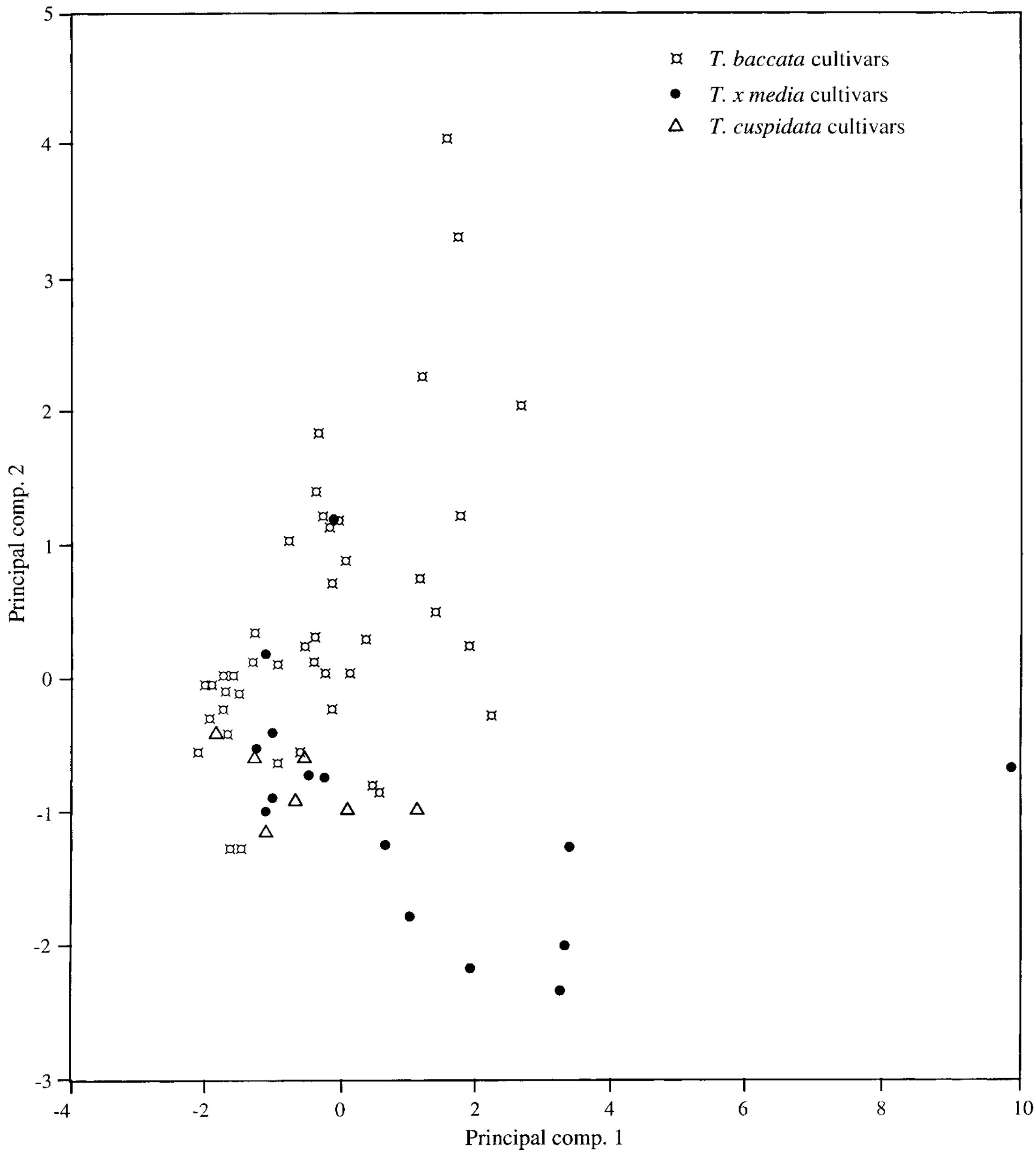

Fig. 5. Principal component analysis based on chemical data for the cultivars of *T. baccata*, *T. x media* and *T. cuspidata*.

T. baccata and *T. x media*. A heterosis effect for the hybrid cultivars is indicated, while the variability is largest in the *T. baccata* cultivars. One *T. x media* cultivar, 'Skalborg', occupies an isolated position, due to its aberrant chemical composition.

3. Experimental

3.1. Morphological data

Morphological characters of 66 cultivars (for which chemical data were also available) were scored and analysed with the software package JMP (SAS Institute Inc., Cary, NC). A cluster analysis, using Ward's clustering criterion, was performed (Fig. 3). The affiliation of the cultivars to species was derived from literature. In case of conflicting sources (many *T. cuspidata* cultivars are listed as *T. x media* cultivars in other handbooks) the hybrid designation was followed.

3.2. Chemical data

About 750 different yew samples were analysed in search for high taxane concentrations. The needles were screened for paclitaxel (**1**), 10-deacetyl paclitaxel (**2**), cephalomannine (**3**), baccatin III (**4**), 10-deacetyl baccatin III (**5**) and brevifoliol (**6**). Their structures and analytical details are given in the preceding paper in this issue. On the basis of these chemical data, cluster analysis (Figs. 1 and 4) and principal component analysis (Figs. 2 and 5) were performed with JMP.

3.3. Chemotaxonomy

The dendrograms based on the morphological and chemical data were compared.

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