ANNONACEAE NEWSLETTER

14

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Annonaceae Newsletter No. 14

Contents:

Annonaceae Workshop, Malaga, 25-27 November 2002

The 12th Flora of Thailand meeting, Bangkok, 25 – 29 November 2002.

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Participants of the Annonaceae Workshop, Malaga 2002. From left to right, front row: Lubbert Westra, Marc Sosef, José Hermoso, José M. Farré, Uwe Scharf, Adriana Lobão, Freek Bakker, Michael Pirie, Lars Chatrou, Dario Grossberger; back row: Angeles Pérez de Oteyza, Ryosuke Oga ta, Carlos López, Iñaki Hormaza, Jifke Koek-Noorman, Paul Maas, Erik Jan van Marle, Roy Erkens, Martin Smeets, Chi -hua Tsou, Heimo Rainer, Johan Mols.

Annonaceae Workshop, Malaga, 25-27 November 2002

Algarrobo-Costa
Estación Experimental La Mayora
Consejo Superior de Investigaciones Cientificas
29750 Malaga (Spain)

The programme

Sunday 24 November 2002

19.30 h Registration at hotel Las Yucas.
20.00 h Informal tour to nearby bars to eat some tapas.

Monday 25 November 2002

9.00 h Welcome by the Director, **Maria Luisa Gomez**.
9.15 h **J.M. Farré:** Announcements.
9.30 h. Morning sessions
- **P.J.M. Maas:** Annonaceae of Acre (Brazil).
- **R.H.J. Erkens:** A Central-American origin for *Guatteria* (Annonaceae) based on 5 chloroplast markers.
- **A. Q. Lobão:** *Guatteria* from Rio de Janeiro State.
- **U. Scharf:** The Genus *Guatteria* in the Guianas.
- **J. B. Mols:** Identifying clades in Asian soursops - molecular phylogeny of *Miliusa* and *Polyalthia* (Annonaceae).
- **M. D. Pirie:** Phylogeny reconstruction in Neotropical taxa of the 'short branch' clade.
- **E.J. van Marle:** Leaf anatomy of paleotropical Annonaceae.
- **L.Y.Th. Westra:** Hand lens aspects of annonaceous wood.

14.00 h Lunch
15.00 h Visit to the cherimoya germplasm bank and Annonaceae collection.

Tuesday 26 November 2002

9.00 h Morning program
- **L.W. Chatrou:** Molecular work in Annonaceae.
- **H. Rainer:** Character progression in *Annona* and their significance for systematic and phytogeographic concepts
- **A. Pérez de Oteyza:** The Spanish cherimoya germplasm bank
- **Chi-hua Tsou:** Variations of anther structure in Annonaceae.
- **J.M. Farré:** Manipulation by prunning of growth and cropping of cherimoya (*Annona cherimola* Mill.).
- **N.Westendorp F.:** Micropropagation of the cherimoya cv. Fino de Jete.
- **C.López E.:** Regeneration and genetic transformation of cherimoya (*Annona cherimola* Mill.).
- **I. Hormaza:** Development of molecular markers for identification and breeding of cherimoya (*Annona cherimola* Mill
- **D. Grossberger:** Cherimoya (*Annona cherimola* Mill.) production studies in California.

14.00 h Lunch
15.00 h Business Meeting
20.00 h Dinner, with the opportunity to taste "jamon iberico" (Iberico ham) and red wine.

Wednesday 27 November 2002
Field Day
Visit to the avocado, mango and other subtropical fruit orchards at Estación Experimental La Mayora.
Followed by travel to Motril (60 km east along the coast) for a visit to cherimoya experiments at Finca Experimental La Nacla.
14.00 h - 15.30 h Lunch
Visit to the old cherimoya plantations and one of the fruit sorting and packing warehouses in Almuñecar - Jete

Figure 1: Cherimoya orchard in Algarrobo-Costa

Figure 2: Estación Experimental La Mayora

Figure 3: Cherimoya orchard near Almuñecar

Figure 4: Cherimoya fruit

Figure 5: Cherimoya tree

Figure 6: Excursion in old Cherimoya orchard

Figure 7: Fruit sorting and packing warehouses in Almuñecar - Jete

Figure 8: Cherimoya fruit ready for transportation

Annonaceae Workshop 2002: report of the general discussion

The chairman thanks all participants for their contributions to the workshop, in particular mr. José M. Farré and colleagues for the smooth organisation and their hospitality.

The main topics to be discussed were:
1. Newsletter
2. Ongoing work & planning
3. Coordination
4. Next meeting

1. Newsletter

Notwithstanding the feeling of nostalgia that this decision brings about, it is decided that the next Newsletter (nr. 14) will be offered as a Web document in stead of the yellow hard copy-version. Abstracts and contributions must be sent to J.M. Farré (tropicalesfasip@terra.es) or J. Koek-Noorman (J.Koek-Noorman@bio.uu.nl) before next Christmas (see also under pt. 3: Website).

2. Ongoing work & planning

L. Chatrou: concentrates on the phylogeny of Annonaceae (whole), mainly on DNA; will visit Costa Rica with Maas and Erkens, 06/2003

A. Lobão: will finish MSc work on *Guatteria*; intends to continue on same, including anatomy, molecular aspects (if possible); will consider about the exact group with the Utrecht people

L. Westra: will start taxonomic revision of *Unonopsis* with Maas (about 25 spp.); the pictures of the wood will be published in some way.

D. Grossberger: producer of Cherimoya; for info: see his website: `http://www.cherimoyas.org/`.

F. Bakker and M. Sosef: are starting up Annonaceae of Africa project in Wageningen, will themselves join after finishing current commitments; the first activity is a revision of *Annickia* by MSc student; in the near future a senior docent and a PhD student will be appointed; *Monanthotaxis* could be another starting point; further suggestions are welcome

M.A. Viruel: aiming to develop new microsatelite markers

J. Koek-Noorman: (after formal retirement, as associate staff member:) intends to carry out some anatomical projects in the scope of research-projects carried out in Utrecht

E.J.Van Marle: hopes to continue with leaf anatomy of Paleotropical Annonaceae

H. Rainer: will finish a PhD-thesis on *Annona*, early next year; will continue with a contribution to Flora of the Guianas; Annonaceae for Flora de Cuba; monograph of *Annona* continued (phytochemistry and wood anatomy still missing)

Chi-hua Tsou : topics are anther development; compound pollen formation; is in need of more material; hopes to link her data with Chatrou's cladogram

Mols: will continue on *Miliusa*, using more DNA sequences; will revise *Miliusa* for Flora Malesiana; a MSc-student will study *Miliusa* of Thailand; MSc student from Taiwan: revision of *Neo-Uvaria*; for Malesia, the large genera remain still to be done, P.Kesler will contribute

Erkens: *Guatteria*, will add nuclear markers to DNA data matrix; small allied genera *Guatteriopsis, Guatteriella, Heteropetalum* will be incorporated

Smeets : would like to find a possibility to do fieldwork in South America

Scharf: is speeding up with *Guatteria* for Flora of the Guianas; will complete PhD thesis next autumn; future commitment uncertain

M. Pirie: concentrates on taxa in 'short branch' clade; *Cremastosperma*; is "in need" of a MSc-student

N. Westendorp and Lopez E.: continuing research on micropropagation and genetic transformation. Lopez E. will do the micrografting as part of the micropropagation.

I. Hormaza will consider joined interest in molecular research with Chatrou

M.A. Pérez de Oteyza: continues work with the Cherimoya germplasm bank

P. Maas: in press/to appear soon: monograph of *Duguetia* (with Westra, Chatrou & collaborators), revision of *Pseudoxandra* (with Westra), Annonaceae for Flora of Saül, and for Flora of Ducke Reserve; revision of *Unonopsis* (with Westra); Annonaceae for Flora of the Guianas (with Rainer: *Annona*; with Scharf: *Guatteria*; with Johnson: *Xylopia*); visit to Costa Rica, Panama (June 2003); will be retired early 2004.

J.M. Farré: remains interested in pollen storage during hot season, the effect on fruit setting; crossfertilization; pruning experiments; germplasm bank (experiencing increasing political problems when trying to acquire material)

3. Coordination

Some points and/or desiderata emerged from the discussions:

There is a general consensus about the need of stronger coordination and cooperation especially with regard to modern systematics/phylogeny. The aim should be to keep people more informed about what is going on, either than to give directions.

The senior lecturer to be appointed in Wageningen, together with Chatrou (Utrecht) and Kesler (Leiden) will form a guidance group, with Chatrou as central person.

Website: Rainer & Chatrou will take care of this, Rainer the technical aspects, Chatrou the contents:

newsletters
short project descriptions (with e-mail adresses, keywords)
indexes: species index for Neotropics (available);
  species index for Asia and Australia (available, provisional);
  species index for Africa (not existing, wanted!);
  type specimens (but see type specimen collection of the NHN -Netherlands);
  DNA material; inventory of
knowledge gaps;
specimen-based database;
noticeboard (modo Taxacom)
manuscripts that are not properly published but valuable for others , i.e. PhD-theses.

4. Next meeting

Next Annonaceae Workshop to be hosted by Vienna, Autumn 2004.

Annonaceae Workshop 2002: texts and abstracts

The family of Annonaceae in the Brazilian state of Acre

Paul J.M. Maas
National Herbarium of the Netherlands – Utrecht University branch

In October 2001 my wife and I had the opportunity to visit the Brazilian state of Acre. Since 1992 the New York Botanical Garden (with as coordinator Dr. Douglas Daly) is executing, in close association with the Federal University of Acre, a project named: **Floristics and Economic Botany of Acre**. That project has two main goals,

1. to make a botanical inventory of the state of Acre and,
2. to establish forest reserves, based on the assembled botanical data.

In order to make a good botanical survey of Acre the project has a close association with lots of plant taxonomists all over the world and so far about 25 were invited to join one of the regular expeditions. These specialists are then asked to join one or more botanical field excursions and to make excellent gatherings of "their own" plant families. They are also asked to assist in teaching young Brazilian students (for example foresters, plant collectors, and biology students) in items like ecology, the taxonomy of their own special group, or in our case to give a small course Neotropical Flora. This proved to be very stimulating for both the teachers and the brazilian students. The project has its home base in Rio Branco, far in the South of Acre, and close to the border with Bolivia. Rio Branco has a small, but nice herbarium (official abbreviation is HPZ), at the edge of an old rubber plantation, and thus surrounded by a beautiful piece of forest.

For those who do not know so much about Brazil: a very large portion of Brazil is the Amazon Region. It it is an immense region and I roughly measured it as 4000 by 2500 km! The Amazon Region includes many countries like Brazil, Venezuela, Colombia, Ecuador, Peru, down to Bolivia in the South. In Brazil several large states, like for example Pará in the East, Amazonas in the middle, and Acre in the west, form a part of the Amazon Region. Acre is the region I will inform you about: it covers an area of about 150.000 km2, and it is about 750 km long and up to 250 km wide. There are 2 big towns in Acre, namely Cruzeiro do Sul in the North West (its population is ca. 65.000) and the capital Rio Branco in the South East (with ca. 250.000 inhabitants). In the whole state of Acre there is only one big road connecting Cruzeiro do Sul with Rio Branco (about 650 km long), but that road is only open to the public during the driest months of the year.

Acre is not very well botanically explored (so far not more than 9 plant collections per 100 km2 ; number for total Brazil and for Venezuela 25; that for Costa Rica 250), and I will mention 2 persons who were active collectors.

The famous **Ernest Ule** was the first one who made plant collections in Acre. His main goal during his field work was the study of rubber producing trees (*Hevea spp.,* but also *Sapium* and representatives of other families). He travelled and collected in Acre between 1901 and 1902, and the main region he visited was the NW, namely the Rio Jurua and its affluents. He collected about 500 nrs., many of which are types.

The second person exploring Acre was the well known taxonomist **Ghillean Prance** who made extensive collections in 1966, and in 1971 (during that last expedition he was assisted by the young Utrecht taxonomist Maas).

The largest collecting activities after the Prance period took place between 1977 and 1987 in the framework of the **Projeto Flora Amazonica**, a project sponsored by the governments of Brazil and the USA. In those 10 years more than 25 botanical, international expeditions took place in Amazonian Brazil, of which three were executed in Acre.

Acre has a quite rich and interesting flora and according to Douglas Daly there occur about 3200 (3273) species. In Acre there is still a lot of forest left and no less than 90% of its surface is still forested. It has by far the lowest rate of deforestation in Brazil. It was also the region of the so-called "seringueiros" people who extracted rubber from the trees of particularly *Hevea brasiliensis*. That extraction was of very high interest and commercial value, mainly in the beginning of 1900, but even up to now rubber is still extracted. The rubber extraction and consequently the conservation of the Amazonian rain forest was much defended by the leader of the seringueiros the famous Chico Mendes, who was finally murdered in 1988.

The state of Acre can be divided into two parts:

1. The NW part, with rich soils and drained by the Rio Juruá and its affluents, like for example the Rio Moa and Rio Juruá-Mirim.
2. The SE part, with poorer soils and drained by the Rio Purus and by the Rio Acre.

1. The NW upper of Acre drained by the Rio Juruá and its affluents is very wet. There is essentially no dry season and there are relatively rich, young soils. The rivers have often a very rich vegetation of palms at their margins. The W frontier with Peru is marked by low mountains (not higher than 600 meters), namely Serra do Moa and Serra do Divisor, which are inhabited by some typically Andean elements. The top of the Serra do Moa which you see here is hardly reachable (as I personally experienced in 1971) as it is covered by a very low, spiny vegetation of terrestrial Bromeliaceae.
Very typical vegetation types in the NW are those on white sands, which occur only at a few places in the upper Rio Juruá. Those white sand vegetations are quite rare in Amazonian Brazil, where they can also be found for example in the Rio Madeira and the Upper Rio Negro.
Some Annonaceae are typical for NW Acre:
-The most common forest type is rain forest on clayish or loamy soils. In that vegetation type we collected ourselves: *Diclinanona calycina* (*Diclinanona* is a small genus with only 3 species, very typical by its fruit which is reduced to only 1 monocarp) and *Guatteria discolor* (one of the few "easy" species in the genus *Guatteria*, nicely characterized by discolorous leaves (leaves with both sides very differently coloured) and by pointed flower buds.
-Species occurring in periodically inundated forests are not common at all in the NW. As only example I give: *Duguetia odorata* (very typical among the Amazonian species of *Duguetia* by its lower leaf side densely covered with peltate hairs).
-A very particular soil type in Acre is the white sands. The only place where we find them in Acre is in the NW. The white sands are covered with low vegetations varying from savannas (where we do not find Annonaceae at all) to somewhat higher vegetations, locally called 'campinara'. Campinaranas are low forests, varying from a few meters up to 15 m tall, with much light reaching the forest floor and with consequently often a quite rich herb layer (with various Monocots, ferns, and lichens). That type of vegetation is not rich in species and is not rich in Annonaceae, but the species found there are often quite special. A first example is: *Anaxagorea brachycarpa* (a typical representative of the genus *Anaxagorea* by its club-shaped, finally explosively dehiscent fruits, but aberrant from all other species in the genus by its midrib which is raised (instead of impressed) on the upper side of the lamina). Another white sand element is *Xylopia multiflora,* a very weird species of that genus with its very elongate and narrow monocarps, not seen in many other Neotropical species of *Xylopia*. An exciting discovery was a species, which we found quite regularly along the margin of a campinarana forest, namely the only collection of an undescribed species of *Guatteria,* characterized by very small leaves (less than 5 cm long) and small flowers and with the inner side of the bark reddish coloured..

2. The SE part of Acre, drained by Rio Purus and Rio Acre has, in contrast to the NW, a pronounced dry season, poorer soils, and it is flat to slightly undulating. Some forests are semi-deciduous and a number of species show affinities to dry forest and savannas, particularly S of the Amazon Region. The floodplains of the Rio Purus are often covered with large grassy vegetations, and as trees often with genera like *Salix* and *Cecropia*, whereas palms are poorly represented.

-Many places in the SE are covered with rain forest, on clayey or loamy soils.

Some of the Annonaceae found there are: *Onychopetalum periquino* (it is locally called "envira cajú", because of its edible fruits which are superficially resembling cajús; *Onychopetalum* is a very small genus with only 3 species, and it is characterized by minute flowers in dense inflorescences, and the number of monocarps reduced to 1). A second species is *Ephedranthus* aff. *amazonicus* **.** *Ephedranthus* is a poorly known small genus with less than 10 species, it can already recognized by its leaf venation (showing sharp angles between midrib and secondary veins) and its flowers are unisexual . A third and last example of a forest species of the SE is a representative of a genus with 3 species only, namely *Cardiopetalum calophyllum.* This species is quite common in the dry cerrados of S Brazil and Bolivia, whereas Acre is the westernmost border of its distribution.

-Like in the NE few species of Annonaceae inhabit periodically inundated rain forests and as only example I include: *Pseudoxandra lucida* (a genus with about 20 species; one of the main characters to distinguish the genus is a vegetative one, namely the presence of a marginal vein which is often very close to the leaf margin).

-White sands do not occur in the SE.

-There are also quite a few Annonaceae which occur all over the state of Acre. Of that group I would like to include the following 3 species inhabiting rain forests: *Rollinia mammifera* , occurring in Amazonian Peru and all over Acre; it is very aberrant from almost all species of *Rollinia* by its almost free fruiting carpels. A second example is *Annona excellens*, a beautiful species of the genus *Annona*, with the lower side of the leaves densely hairy and with quite large flowers . A third species is a very spectacular one, with very long pedicels of up to over 20 cm long and with boat-shaped inner petals, namely *Cymbopetalum longipes*.

-One of the few species found in periodically inundated forests all over Acre, but also all over tropical South America is *Annona hypoglauca*, very easily distinguished by its leaves which have a glaucous lower side; its spiny fruit is according to Gottsberger fish-dispersed.

In the following figure the number of species occurring in the various regions of Acre are summarized:

Throughout the whole state of Acre thus both in the NW and the SE: 23 spp. of Annonaceae, of which 19 occur in non-inundated forests, and 4 in periodically inundated forests.

There are 30 spp. of Annonaceae exclusively occurring in non-inundated forest of the NW, and 37 spp. occurring exclusively in the SE of Acre. The number of species in inundated forests is also almost equal (2 versus 3) for both the NW and SE. The only important difference is the the white sand species, of which there are 5 in the NW, whereas there are no white sands in the SE.

As conclusion: both regions, the NW with its Rio Juruá and the SE with its Rio Purus and affluents, have their own, quite distinct flora with each about 40 different species of Annonaceae, whereas they have 23 species in common. Counting from the table we see that Acre with its 100 species of Annonaceae is not quite rich (but this number will probably rise to about 150 after more intensive exploration). There are hardly any endemic Annonaceae in Acre, except for the mini-leaved species of *Guatteria.*

The number of species of Annonaceae is by far the highest in Peru, namely almost 200 (probably caused by the fact that the country is very large, but also that it includes mountain ranges like the Andes). Surprisingly, the number of genera of Annonaceae is the highest in Acre (no less than 26). This is probably due to the fact that Acre not only includes Amazonian species, but also species coming from the dry cerrados in the South, that it has some mountain ranges, and that white sands are found.

A last remark about forest conservation in Acre: the most interesting regions, regions which would merit the status of forest reserves are the white sand vegetations with their spectacular and unique campinaras as. found in the NW.

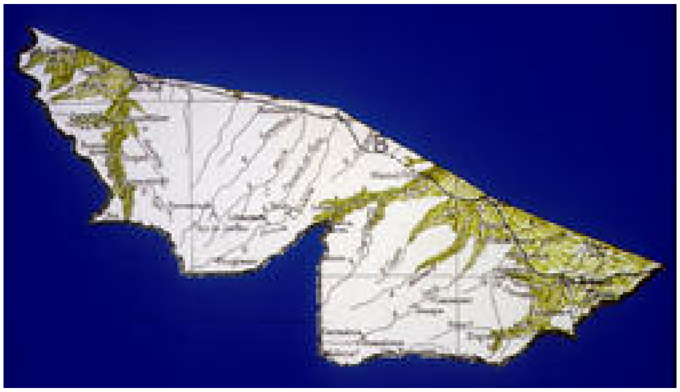

Figure 1: Acre, Brazil, showing Rio Branco in the SE and Cruzeiro do Sul in the MNW.

Figure 2: Acre, Brazil.

Figure 3: Rio Moa.

Figure 4: Serra do Moa (slide from 1971).

Figure 5: Xylopia multiflora, Maas et al. 9200.

Figure 6: Guatteria sp. nov., Maas et al. 8980.

Figure 7: Rio Purus (slide from 1971).

Figure 8: Onychopetalum periquino, Maas et al. 9255.

Figure 9: Cardiopetalum calophyllum, Maas et al. 8895.

Figure 10: Annona excellens, Maas et al. 9272.

Figure 11: Annona excellens, Maas et al. 9272, opened flower.

Figure 12: Xylopia sp., Maas et al. 9034.

Figure 13: Number of genera and species of Annonaceae in South America.

A Central-American origin for *Guatteria* (Annonaceae) based on 5 chloroplast markers

Roy H.J. Erkens
National Herbarium of the Netherlands – Utrecht University branch

The Neotropical genus *Guatteria* is one of the largest woody plant genera in the world and the largest within the family of Annonaceae, comprising approximately 260 species. Revision of this genus dates back to 1939, when Fries made a treatment based on few macromorpholocial characters. He divided the genus into 30 sections. Up to now it is the only major genus of Neotropical Annonaceae that awaits revision. Because of its homogeneity in many morphological characters it is very difficult to understand the systematics and evolution of the genus. The lack of a taxonomic framework and of a classification of the genus severely hampers the evaluation of the large quantities of new material that have been collected throughout recent years. This research started to unravel the classification of *Guatteria* by using molecular markers.

In this study a parsimony analysis was performed on 47 taxa of *Guatteria* using DNA sequence data of the plastid genome (*rbcL* region, *matK* region, *trnL-F* spacer and *trnL* intron and *psbA-trnH* spacer). Although these data are still insufficient to resolve the relationships between all closely related species, they do show that *Guatteria* is a very well supported monophyletic group. Furthermore, the genus seems to have its origin in Central-America, rather than in South-America.

Guatteria from Rio de Janeiro

Adriana Lobão
Univ. de São Paulo, Inst. de Biosciências; São Paulo SP, Brazil

The genus *Guatteria* is characterized by leaves with impressed primary vein on the upper side; pedicels with suprabasal articulation; indument of simple hairs, flowers mostly solitary, axillary, sepals free, petals free, green, cream to yellow, inner ones imbricate, fruit apocarpous, monocarps many, indehiscent, 1 -seeded.
In number of species *Guatteria* is the biggest genus from Annonaceae and the most problematical one. Because of the misinterpretation of flower maturation, the similarity of reproductive characters among species and the great morphological variation in vegetative characters. Many reseachers described a large number of species because all these points could have confused them.

Having all these points in mind, the aim of these work was to make a survey on *Guatteria* species in Rio de Janeiro State in order to know the main features to distinguish species. As a result, 12 species were recognized in Rio de Janeiro. The majority occur in hill forest, normally near rivers.

In Rio de Janeiro State the majority of the reproductive characters from *Guatteria* species were not informative; however, some of them were important to distinguish species. A very important feature was the fusion of the sepals in the flower bud. In Rio de Janeiro, the species may have free or connate sepals in flower bud. Other reproductive character used were the number of flowers. The flowers may be solitary, 2 or placed in many flowered inflorescences on the trunk. The last important reproductive character used was the shape of petals. Some species have narrow petal with acute apex and others have oblong petals with obtuse apex.

Vegetative characters were used only in association with others.

The work is not finished yet and we keep looking for useful features to distinguish species; therefore, we've been working on anatomical studies. Also, I've been visiting herbaria where types of these species are and I hope that helps me solve some problems of *Guatteria* taxonomy.

The genus *Guatteria* (Annonaceae) in the Guianas.

Uwe Scharf
Leipzig University, Germany and National Herbarium of the Netherlands, Utrecht University branch

As part of both the Annonaceae and the Flora of the Guianas projects a regional revision of the genus *Guatteria* for the Guianas (French Guiana, Guyana, Suriname) is in progress. This PhD was started in Utrecht under the supervision of Prof. P. Maas (Utrecht) and Prof. W. Morawetz (Leipzig) two years ago. The overwhelming majority of the collections as well as the most active workgroup in the subject are located in Utrecht. Work with herbarium sheets yielded the first and most results. More than 80 % of the collections belong to less than 10 % of the species, including species-complexes. New species for the Guianas are almost always from remote areas and poorly collected.

A field study of seven weeks was carried out in September/October 2001 in all three Guianas as a collaboration between the herbaria in Leipzig (LZ) and Utrecht (U) and the national herbaria in Cayenne (CAY), Georgetown (BRG) and Paramaribo (BBS). Triplicate collections were made for the national herbaria and U and LZ. Of approximately 100 collections, 82 belong to the Annonaceae family, 32 to seven species of the genus Guatteria. All these collections were collected both as herbarium material and as samples on silica gel for molecular research. The trip contributed enormously to the understanding of Annonaceae, especially of Guatteria, their environment and flower biology.

An overview of the project and preliminary results can now be presented. To date, of the more than 40 names in the collections from the Guianas, 17 have been confirmed, six new species have been described, one name has been reinstated to and one name removed from the list of the region, and at least 11 names have been declared as synonyms. Four difficult species-complexes have been recognised (G. atra, G. brachypoda, G. punctata, G. schomburgkiana). Their resolution (treatment) has been postponed.

At the end of 2002 a first manuscript of the species descriptions will be submitted in Utrecht. In February 2003 the next workshop of the FoG project in Cayenne will be attended, and field trips in French Guiana will be undertaken. The thesis will be submitted in Leipzig in late autumn 2003, and defended at the end of the year.

Identifying clades in Asian soursops - molecular phylogeny of *Miliusa* and *Polyalthia* (Annonaceae).

Johan B. Mols, Paul J.A. Kessler & Barbara Gravendeel
National Herbarium of the Netherlands, Leiden University branch

The Annonaceae are a pantropical family of trees, shrubs and lianas consisting of ca. 130 genera and 2300 species. Although the family is clearly defined, generic and tribal delimitation of especially the Asian Annonaceae is controversial due to many overlapping morphological characters. The main goal of this study was to reconstruct a molecular phylogeny of the genus *Miliusa*, which is traditionally assigned to the tribe Miliuseae together with five other genera, and of the genus *Polyalthia* which is generally regarded to be a polyphyletic genus.

Parsimony analysis of cpDNA *rbc*L, *trn*L intron and *trn*L-*trn*F intergenic spacer sequence data, collected from more than 100 taxa occurring in Asia, Africa and America, was performed. *Miliusa* proved to be monophyletic, just as three other genera in the Miliuseae (*Alphonsea*, *Orophea* and *Platymitra*). Separate clades in *Miliusa* will be correlated with morphological and geographical patterns. *Polyalthia* proved to be highly polyphyletic, with species ending up in different clades. Options for a phylogenetically based subdivision of *Polyalthia* into several smaller genera will be discussed.

Figure 1: Section *Miliusa*: *M. thorelii* (© Chalermglin)

Figure 2: Section *Saccopetalum: M. horsfieldii* (© Chalermglin)

Figure 3: Third *Miliusa* clade*: M. amplexicaulis* (© Chalermglin)

Annonaceae: Phylogeny reconstruction in Neotropical taxa of the 'short branch' clade

Michael D. Pirie[1], Johan B. Mols[2] & Lars W. Chatrou[1]
[1]National Herbarium of the Netherlands, Utrecht University branch,
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Projects at the National Herbarium of the Netherlands are contributing to the building of a molecular phylogeny of the family Annonaceae. Results so far divide the family into a number of basal lineages plus two major derived clades with contrasting apparent rates of molecular evolution. The consequences for reconstructing phylogeny in taxa of the 'short branch' clade is a far greater demand for character sampling.

Supporting monophyly of genera and their closest sister groups for use in species level phylogeny remains challenging. This is the particular aim of my PhD project, with respect to the Neotropical genera *Cremastosperma*, *Malmea*, *Mosannona* and *Klarobelia*. With the markers *rbc*L and *trn*L-F these genera fall within three separate clades, the relationships between which are unresolved in a basal polytomy also including a contrastingly well supported large clade of almost exclusively Asian distribution. Relationships at this level could thus be key to the understanding of biogeographical relationships within the clade as a whole.

Preliminary results of analyses using a range of chloroplast markers at species level in *Cremastosperma* and at generic level will be presented, and biogeographic implications discussed.

Leaf anatomy within the Annonaceae of the old world. A preliminary survey.

E.J.van Marle
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Leaf anatomy of the Annonaceae of the Neotropics has been studied by A.K.van Setten in 1984. To continue this study I started with a survey of the genera from the rest of the world. The aim of this research is to see if it is possible to find characters which are specific to recognize genera or groups of genera.
From the 92 collected genera 88 have been looked at, representing 134 species. Over 500 collections are waiting for further treatment.

Preliminary conclusions
All the characters found are in agreement with those found in the Neotropical genera.
Until now it is hardly possible to come to groupings of genera with the present available information. Taxonomical groupings known from the literature are not supported by my data. With one exception. One group of genera, recognized by Setten van and Koek-Noorman (1992), is distinguished by the presence of very large crystals in the adaxial epidermis. In *Disepalum*, a genus also accommodated in this group by Koek et al. (1997), these large crystals are lacking. However, the group, most representatives of which are climbers, doesn't show very clearly in their cluster analysis.
The large crystals, which are such a striking feature, are apart from this group, only found in the genus *Exellia*.
Round osteosclereids are only found in one genus, that of *Xylopia*. A layer of flattened parenchyma cells that extends at both sides of the midvein into the lamina in *Monodora*. Irregular shaped vascular systems in *Boutiquea, Dasymaschalon* and *Mitrella*.

References

Koek-Noorman, J.; A.K. van Setten & C.M. van Zuilen 1997. Studies in Annonaceae XXVI. Flower and fruit morphology in Annonaceae. Their contribution to patterns in cluster analysis. Bot. Jahrb. Syst. 119(2): 213-230.
Setten, A.K. van 1984. Bladanatomy van de Annonaceae (m.n. de neotropische genera). Studentreport.
Setten, A.K. van and J. Koek-Noorman 1986. Studies in Annonaceae VI. A leafanatomical survey of genera of Annonaceae in the Neotropics. Bot. Jahrb. Syst. 108(1): 17-50.
Setten. A.K. van and J. Koek-Noorman 1992. Fruits and seeds of Annonaceae. Morphology and its significance for classification and identification. Studies in Annonaceae XVII. Biblioth. Bot. 142: 1-101, pl. 1-50.
Zuilen, C.M. van 1996. Patterns and affinities in the Duguetia alliance (Annonaceae). Molecular and morphological studies. Thesis.

Hand Lens Aspects of Annonaceous Wood

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(As this contribution greatly depended on the slide presentation, it was decided to incorporate the complete text and illustrations)

Introduction

Annonaceae have a characteristic wood structure. Two important features should be mentioned here: first, the large rays and, secondly, tangential apotracheal parenchyma bands. Both contribute to the cobweb -like appearance of annonaceous wood in cross-section (end grain), even to the naked eye. Anything like it is not or hardly known in other families leaving the Annonaceae " unique in this respect.

As for the infra-familial level, annonaceous wood reportedly is quite homogeneous, i.e., variation such as can be observed seems to give little support to (= only weakly correlated to) classifications on, e.g., tribal or generic level. Microscopical investigations in several (Neotropical) genera by Ter Welle during the past twenty years indicate that occasionally a genus or perhaps a group of genera in the sense of R.E. Fries (1959) may stand out by a unique feature or combination of character states, but in other cases it is at best one or more individual species that could be distinguished by the wood (Ter Welle, 1984a/b, 1985, 1992, 1995, 1997; Ter Welle & Van Rooden, 1982).

Nevertheless, given the variation that is definitely there notably in cross-sectional view, the wood might still be of help in identification also for the taxonomist working on the gross morphological level but equipped with a good hand lens. Some pictures may serve as examples here. These end grain photographs are comparable to, among others, those published by Lindeman & Mennega some time ago (Lindeman & Mennega, 1963), or more recently by Ilic (1991).

Material and method

Small wood blocks that previously had been cut so size in order to facilitate microtome sectioning were used for photography. The flat surface left after the cutting of microtome slices was photographed using a Zeiss Luminar 40 mm f/4.5 macro objective (stopped down 1/2 stop) on Kodachrome 25 film. All photographs were taken at a repro ratio of 5:1 on the 35 mm frame. Thus the images represent a rectangle of ± 7x4.5 mm.

Iconics

1. The Annona/Rollinia complex

Examples: *Annona* cf. *densicoma* (Fig. 1), *A. montana* (Fig. 2), *A. sericea* (Fig. 3), *Rollinia edulis* (Fig. 4), *R. exsucca* (Fig. 5), *R. mucosa* (Fig. 6). In *A.* cf. *densicoma* and *A. montana* rays vary from narrow to (particularly in *A. montana*) broad, while *A. sericea* shows only narrow rays. Vessels in *A. montana* are rather large and mostly solitary, and similar (but even larger) in *A. sericea*. *A.* cf *densicoma* has distinctly smaller vessels and mostly in radial arrangement. The banding pattern is relatively fine in *A.* cf. *densicoma*, but coarse in *A. montana* and *A. sericea*. In *A. montana*, moreover, the parenchyma bands are not very distinct from the fibres, hence not easy to spot. The three *Rollinia* species, like the two preceding Annnonas, have rather large and mostly solitary vessels (largest in *R. exsucca*). The rays are narrow in *R. exsucca*, and more variable in *R. edulis* and *R. mucosa*. The banding is rather coarse to very coarse in all three species, with (seasonal) variations in *R. edulis*, and quite indistinct in *R. exsucca*.

2. Anaxagorea

Examples: *Anaxagorea acuminata* (Fig. 7), *A. dolichocarpa* (Fig. 8). The rays vary from narrow to broad, very broad in *A. dolichocarpa*, and tend to be relatively wide apart. Both species show quite small vessels often in radial arrangement, and tend toward a fine banding pattern.

The tangential section of *A. dolichocarpa* (Fig. 9) shows the chararistic reticulate pattern due the broad rays; this also accounts for the reticulate macroscopic aspect of the bark both here and in many other members of the Annonaceae. In radial view the parenchyma bands stand out as fine longitudinal streaks (Fig. 10).

3. Guatteria

Examples: *Guatteria chrysopetala* (Fig. 11), *G. schomburgkiana* (Fig. 12), *G. trichostemon* (Fig. 13). Rays tend to be mostly rather broad, and rather close together. Vessels are generally large and mostly (especially the largest ones) solitary. The banding is (often) rather coarse.

4. Cremastosperma alliance

Examples: *Cremastosperma cauliflorum* (Fig. 14), *C. microcarpum* (Fig. 15), *Ephedranthus guianensis* (Fig. 16), *Klarobelia megalocarpa* (Fig. 17), *Malmea dielsiana* (Fig. 18), *M. obovata* (Fig. 19), *Mosannona* aff. *discolor* (Fig. 20), *M. pacifica* (Fig. 21), *M. raimondii* (Fig. 22), *Pseudomalmea diclina* (Fig. 23), *Oxandra asbeckii* (Fig. 24), *O. riedeliana* (Fig. 25), *Pseudoxandra polyphleba* (Fig. 26), *Bocageopsis multiflora* (Fig. 27), *Unonopsis glaucopetala* (Fig. 28), *U. guatterioides* (Fig. 29), *U. perrottetii* (Fig. 30), *U. rufescens* (Fig. 31). The rays mostly tend to be narrow with occasionally some broader ones in between; broad rays occur in *Cremastosperma*, especially *C. cauliflorum*, in *Bocageopsis*, and in three of the *Unonopsis* species (not in *U. perrottetii*). Vessels, solitary or in radial arrangement, appear to be small in most cases, somewhat larger in *Cremastosperma cauliflorum*, *Ephedranthus guianensis*, *Pseudomalmea diclina*, *Oxandra riedeliana*, *Pseudoxandra polyphleba*; however, the "big look" is caused in part by the spacing of the rays, notably in *Ephedranthus guianensis* where the rays are so close as to often touch the vessels on both sides. Really large-looking vessels appear in *Bocageopsis*, and in *Unonopsis glaucopetala*, *U. guatterioides* and *U. rufescens*. *U. perrottetii* stands somewhat apart by the smaller vessels and, as indicated before, by the narrower rays. A (rather) fine banding prevails in most of the genera (indistinct in *Pseudomalmea diclina*). In *Bocageopsis* and *Unonopsis* the banding is rather coarse.

5. Xylopia

Examples: *Xylopia aromatica* (Fig. 32), *X. benthamii* (Fig. 34), *X. frutescens* (Fig. 35), *X. cuspidata* (Fig. 36), *X. peruviana* (Fig. 37). Rays are narrow and quite close in *X. aromatica*, *X. benthamii* and *X. frutescens*; in *X. peruviana* there tend to emerge somewhat broader and more distant rays between the narrow ones. In *X. cuspidata* the rays are markedly broader and more distant. Vessels are large in *X. aromatica* and *X. frutescens*, solitary or in radial arrangement, and often touching rays on both sides, in *X. benthamii* they are somewhat smaller but still tending to touch rays. *X cuspidata* and *X. peruviana* have small vessels, solitary or in radial arrangement. Banding is rather coarse in *X. aromatica*, *X. benthamii* and *X. frutescens*, distinctly finer in *X. peruviana* and particularly *X. cuspidata*.

Note the narrow rays of *Xylopia aromatica* also in tangential section (Fig. 33). The reticulate pattern as present in, e.g., *Anaxagorea* (Fig. 10) is much less obvious here, and also in the corresponding bark on the outside.

6. Duguetia

Examples: *Duguetia cadaverica* (Fig. 38), *D. calycina* (Fig. 39), *D. neglecta* (Fig. 40), *D. odorata* (Fig. 41), *D. staudtii* (Fig. 42). Rays are broad to very broad in *D. cadaverica*, from narrow to rather broad in *D. calycina*, *D. neglecta* and *D. staudtii* (with an occasional very broad ray in between in *D. calycina*); in *D. odorata*, in contrast, the rays are rather narrow and close together. Vessels are very small and mostly in radial arrangement in *D. cadaverica*, in *D. calycina*, *D. neglecta* and *D. odorata* they are small to medium in size, mostly in radial arrangement too. *D. staudtii* has rather large vessels, solitary or in radial arrangement. The banding is very fine in *D. cadaverica*, and fine to rather coarse in the other species.

7. Lianas

Examples: *Annona haematantha* (Fig. 43), *Friesodielsia soyauxii* (Fig. 44), *Toussaintia hallei* (Fig. 45). Adaptation is effected through enlargement of vessel diameter; in *Annona haematantha* (New World) the transition is more or less gradual, in the two African examples much more abrupt, particularly in *Toussaintia hallei* (*Friesodielsia soyauxii*, in turn, has very wide vessels); note that tangential parenchyma bands tend to become indistinct (" disappear from view) in liana wood.

Discussion

The *Annona*/*Rollinia* complex is variable, and this is reflected in the variation in the wood which largely seems to coincide with that of the family as a whole. Wood of *Anaxagorea*, on the other hand, seems much more uniform, characterized by frequent presence of broad rays rather wide apart, and small vessels often in radial arrangement. Wood of *Guatteria*, another example of a rather uniform appearance, shows the rays rather close to each other, in combination with vessels of quite large diameter and often solitary. In the *Cremastosperma* alliance there seems to be a tendency toward rather narrow rays and not-too-large vessels; *Unonopsis* and, to a lesser extent, *Bocageopsis* (and perhaps *Ephedranthus*) seem exceptions here. Within *Unonopsis* the somewhat aberrant look of *U. perrottetii* is remarkable, as this species also macromorphologically stands more or less apart from other *Unonopsis* species (Maas, pers. comm.). *Xylopia* seems to fall apart into two groups, one with large vessels and rather closely spaced rays, and the other with small vessels and more widely spaced rays C giving an impression of finer grain. The species seen in the first group have flowers with long petals, and are shrubs to trees, while those in the second group have comparatively broad petals, and are (small) shrubs. *Duguetia*, while easily recognized by, among others, leaf characteristics, seems very variable in the wood. Even so, as for the wood, the African species *D. staudtii* looks quite different from the Neotropical representatives seen. It might be interesting here to view the wood of the three other African species (all formerly referred to *Pachypodanthium*) as well.

Adaptation to climbing in Annonaceae seems to come through enlargement of vessel diameter. So far, no abnormal cambial activity, or bark intrusions, etc., well known from lianas in a number of other families, seem to have been recorded.

Conclusion

While it probably goes too far to expect wood features uniquely restricted to genera or groupings at other levels (this applies to aspects visible by hand lens we are dealing with here, but will equally be true regarding the microscope), wood, on the other hand, should not be dismissed as non-informative either. Rather, we could value wood characteristics as we do with, e.g., leaf characteristics. Whenever possible, plant descriptions should also include hand lens aspects of the wood.

Acknowledgment

The authors are very thankful to Dr. Alberta M.W. Mennega for her advice in fruitful discussions we had with her, and for her warm support.

References

Fries, R.E. 1959. Annonaceae. In: Melchior, H. (ed.) C Die Natürlichen Pflanzenfamilien ... begründet von A. Engler und K. Prantl, ed. 2, Band 17 a II: 1-171. Berlin: Duncker & Humblot.

Ilic, J. 1991. CSIRO atlas of hardwoods. Berlin [etc.]: Springer Verlag.

Lindeman, J.C. & A.M.W. Mennega. 1963. Bomenboek voor Suriname. Paramaribo: Dienst 's Lands Bosbeheer.

Welle, B.J.H. ter 1984a. Variation and diversity in the Annonaceae. Proceedings of Pacific regional wood anatomy conference: 144-146. Ibaraki 305 (Japan), Wood Technol. Div. Forestry and Forest Products Res. Inst.

-- 1984b. Wood anatomy. In: Maas, P.J.M. & L.Y.Th. Westra C Studies in Annonaceae II. A monograph of the genus Anaxagorea A.St.Hil., Part 1. Bot. Jahrb. Syst. 105: 82 -84.

-- 1985. Wood anatomy. In: Westra, L.Y.Th. C Studies in Annonaceae IV. A taxonomic revision of Tetrameranthus R.E.Fries. Proc. Kon. Nederl. Akad. Wetensch. C 88: 455-456.

-- 1992. Wood anatomy. In: Maas, P.J.M., L.Y.Th. Westra & Collaborators - Rollinia. Fl. Neotrop. Monogr. 57: 21-26.

-- 1995. Wood anatomy. In: Westra, L.Y.Th. - Studies in Annonaceae XXIV. A taxonomic revision of Raimondia Safford. Bot. Jahrb. Syst. 117: 276 -278.

-- 1997. Wood anatomy. In: He, Ping & L.W. Chatrou C Studies in Annonaceae XXXIII. A monograph of Fusaea (Baill.) Saff. In: He, Ping C Neotropical Annonaceae. Studies in morphology, taxonomy, and phylogeny: 140-142. Utrecht University (Thesis).

Welle, B.J.H. ter & J. van Rooden 1982. Systematic wood anatomy of Desmopsis, Sapranthus and Stenanona (Annonaceae). IAWA Bull. 3: 15 -23.

Fig. 1. *Annona* cf. *densicoma* (Uw 4012), cross section.

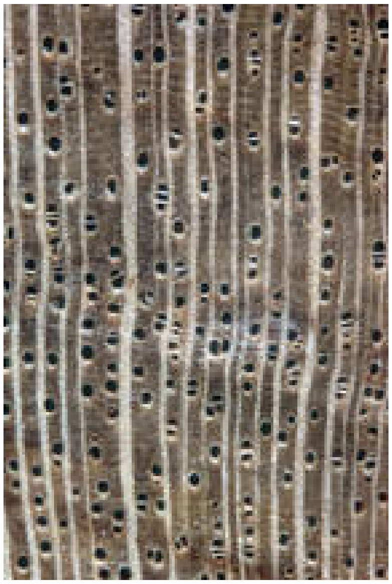

Fig. 2. *Annona montana* (Uw 1465), cross section.

Fig. 3. *Annona sericea* (Uw 9126), cross section.

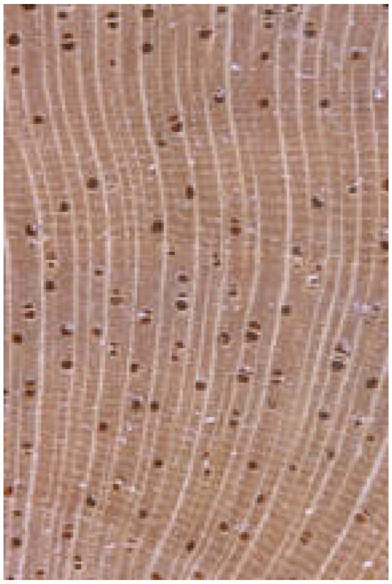

Fig. 4. *Rollinia edulis* (Uw 30324), cross section

Fig. 5. *Rollinia exsucca* (Uw 26580), cross section

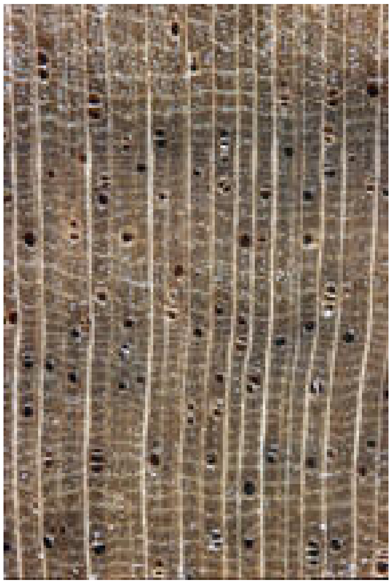

Fig. 6. *Rollinia mucosa* (Uw 30239), cross section.

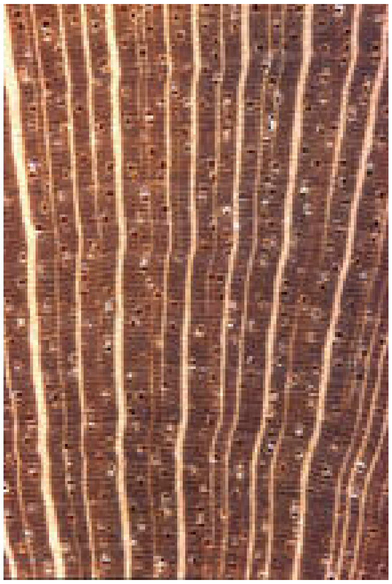

Fig. 7. *Anaxagorea acuminata* (Uw 19208), cross section.

Fig. 8. *Anaxagorea dolichocarpa* (Uw 19575), cross section.

Fig. 9. *Anaxagorea dolichocarpa* (Uw 19575), tangential lg. section.

Fig. 10. *Anaxagorea dolichocarpa* (Uw 19575), radial lg. section.

Fig. 11. *Guatteria chrysopetala* (Uw 2565), cross section.

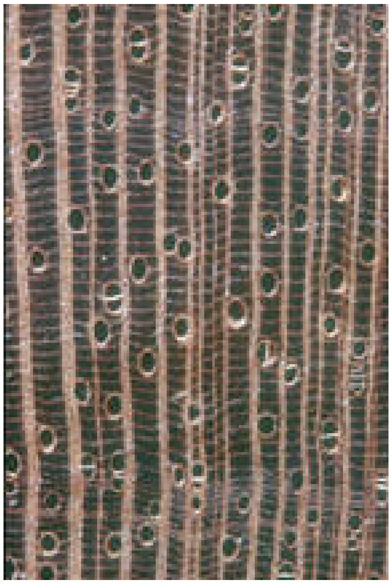

Fig. 12. *Guatteria schomburgkiana* (Uw 267), cross section.

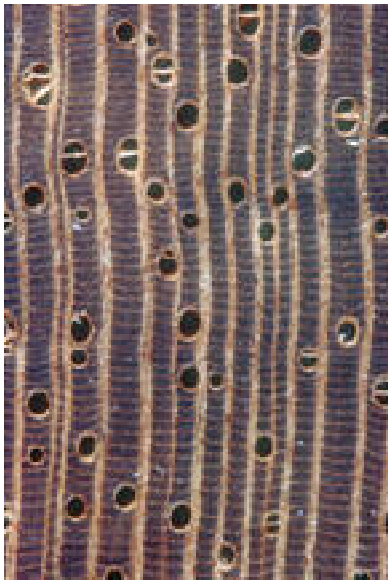

Fig. 13. *Guatteria trichostemon* (Uw 16119, from type collection), cross section.

Fig. 14. *Cremastosperma cauliflorum* (Uw 30306), cross section.

Fig. 15. *Cremastosperma microcarpum* (Uw 34858), cross section.

Fig. 16. *Ephedranthus guianensis* (Uw 6856), cross section.

Fig. 17. *Klarobelia megalocarpa* (Uw 35946), cross section.

Fig. 18. *Malmea dielsiana* (Uw 30246), cross section.

Fig. 19. *Malmea obovata* (Uw 8543), cross section.

Fig. 20. *Mosannona* aff. *discolor* (Uw 26395), cross section.

Fig. 21. *Mosannona pacifica* (Uw 35947), cross section.

Fig. 22. *Mosannona raimondii* (Uw 34863), cross section.

Fig. 23. *Pseudomalmea diclina* (Uw 20084), cross section.

Fig. 24. *Oxandra asbeckii* (Uw 271), cross section.

Fig. 25. *Oxandra riedeliana* (Uw 7793), cross section.

Fig. 26. *Pseudoxandra polyphleba* (Uw 19657), cross section.

Fig. 27. *Bocageopsis multiflora* (Uw 6796), cross section.

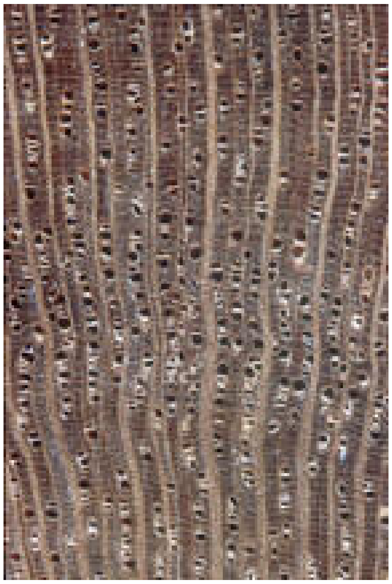

Fig. 28. *Unonopsis glaucopetala* (Uw 2317), cross section.

Fig. 29. *Unonopsis guatterioides* (Uw 370), cross section.

Fig. 30. *Unonopsis perrottetii* (Uw 772), cross section.

Fig. 31. *Unonopsis rufescens* (Uw 225), cross section.

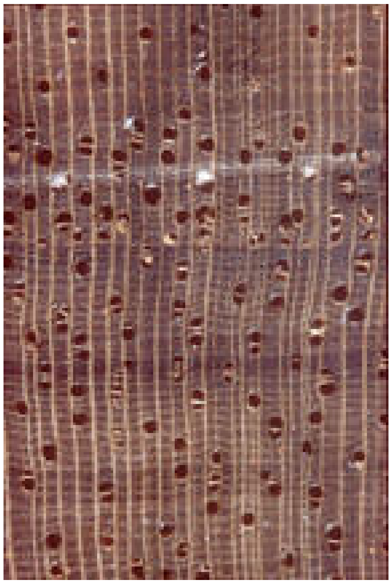

Fig. 32. *Xylopia aromatica* (Uw 7762), cross section.

Fig. 33. *Xylopia aromatica* (Uw 7762), tangential lg. section.

Fig. 34. *Xylopia benthamii* (Uw 9071), cross section.

Fig. 35. *Xylopia frutescens* (Uw 775), cross section.

Fig. 36. *Xylopia cuspidata* (Uw 30325), cross section.

Fig. 37. *Xylopia peruviana* (Uw 30226), cross section.

Fig. 38. *Duguetia cadaverica* (Uw 8611), cross section.

Fig. 39. *Duguetia calycina* (Uw 15331), cross section.

Fig. 40. *Duguetia neglecta* (Uw 27370), cross section.

Fig. 41. *Duguetia odorata* (Uw 19628), cross section.

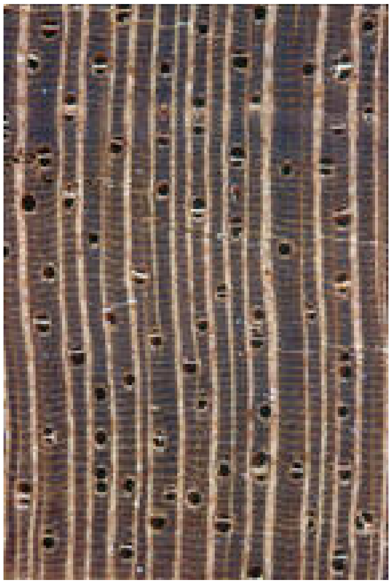

Fig. 42. *Duguetia staudtii* (Uw 6562), cross section.

Fig. 43. *Annona haematantha* (Uw 2572), cross section.

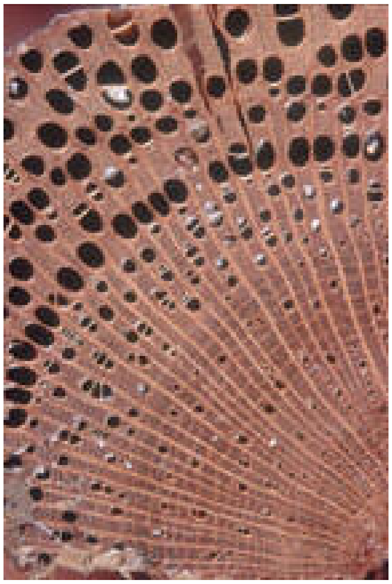

Fig. 44. *Friesodielsia soyauxii* (Uw 29519), cross section.

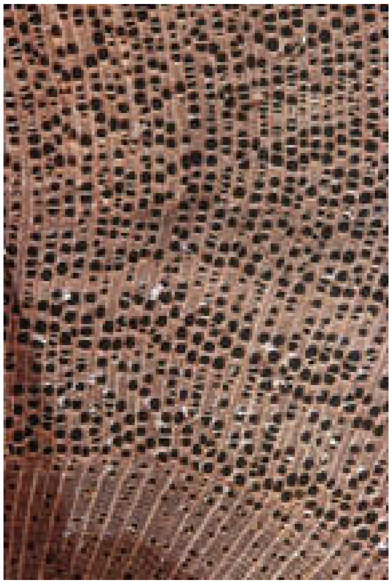

Fig. 45. *Toussaintia hallei* (Uw 29546), cross section.

Species-level phylogenetics in Neotropical Annonaceae

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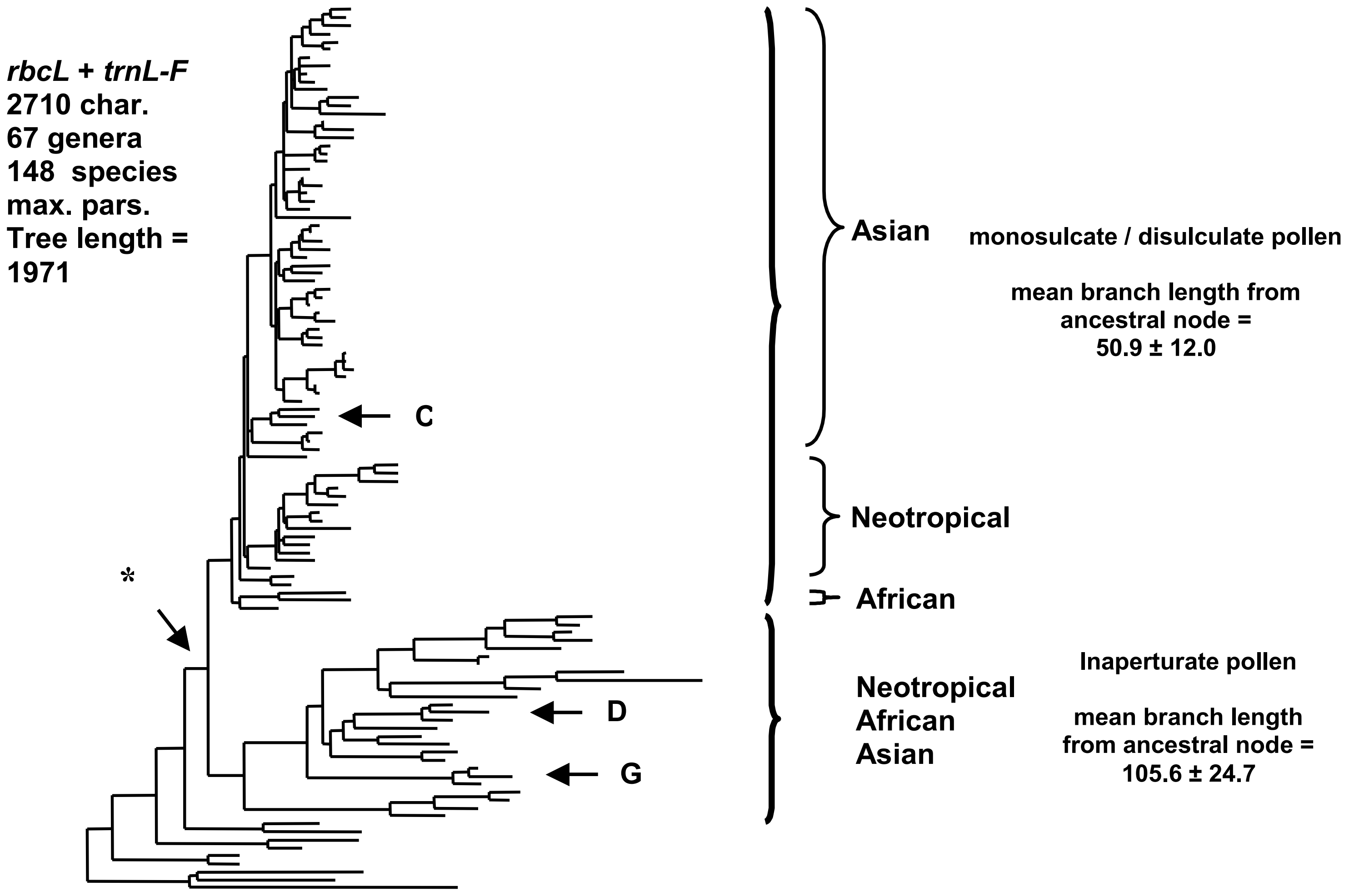
Neotropical representatives of the Annonaceae have been a major focus of systematic research at the Utrecht branch of the National Herbarium of the Netherlands since the founding of the international Annonaceae project 17 years ago. Extensive revisionary work and the accumulation of numerous new collections has been complemented in recent years by the building of a phylogeny of the family as a whole. Despite the difficulty of adequately sampling with respect to species level phylogeny reconstruction, sufficient material has been collected to resolve species-level phylogenies of a number of Neotropical genera.

Species-level projects are in progress on *Duguetia*, *Guatteria*, and *Cremastosperma*. Phylogenies of the three genera are reviewed in the context of the family phylogeny of the Annonaceae. Fig. 1 shows a phylogram of 148 species of Annonaceae, representing 67 genera. Genera are represented by no more than three species. With the exception of a few basal lineages, the majority of the species diverge into two major sister clades, distinct on the basis of pollen morphology, geographic distribution, and patterns of molecular evolution. The point of divergence between the two clades is indicated by an asterisk. One of these clades is characterised by inaperturate pollen and has an apparent rate of molecular divergence roughly twice the rate of the other clade, characterised by monosulcate and disulculate pollen (see Fig. 1). Rates of molecular divergence for both clades were inferred by taking the mean of branch lengths from the most recent common ancestor to all terminals.

Duguetia (the three representative species in Fig. 1 are indicated by an arrow and "D") and *Guatteria* (arrow and "G") are in the long-branch-clade, *Cremastosperma* (arrow and "C") is in the short-branch-clade. For our species-level phylogenies we increased both taxon and character sampling relative to the sampling for the family phylogeny. For *Cremastosperma* 30 out of 35 species are included in our analysis, 35 out of 96 species of *Duguetia*, and 50 out of ca. 250 species of *Guatteria*. For each of the genera phylogenies are presented on the basis of the following cpDNA markers: *rbcL*, *trnT-L* intergenic spacer, *trnL* intron, , *trnL-F* intergenic spacer, *psbA-trnH* intergenic spacer, and partial *matK*.

Mean branch length from the ancestral node to the terminals for the three genera separately show that *Guatteria* has the highest levels of divergence (49.6 ± 13.4), and differs significantly from the levels of divergence present in *Duguetia* (26.3 ± 6.3) and *Cremastosperma* (17.7 ± 6.2). Rates of divergence between the latter two genera do not differ significantly. The higher levels of divergence of *Guatteria* correspond to better resolved trees (maximum parsimony) and more nodes that are well supported by bootstrap percentages for this genus, compared to trees of *Duguetia* and *Cremastosperma*.


rbcL + trnL-F
2710 char.
67 genera
148 species
max. pars.
Tree length =
1971
*
C
D
G
Asian
monosulcate / disulculate pollen
mean branch length from
ancestral node =
50.9 ± 12.0
Neotropical
African
Neotropical
African
Asian
Inaperturate pollen
mean branch length
from ancestral node =
105.6 ± 24.7

The Spanish Cherimoya (*Annona cherimola Mill.*) Germplasm Bank

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The Spanish Germplasm Bank was started in 1979 – 1985 as a small commercial cultivar collection. From 1986 it has grown steadily. Most of the material has been collected in the main Andean countries, Peru, Ecuador and Bolivia, where Annona fruit is produced. Besides, collections have been acquired by exchange from most of the remaining cherimoya producing countries. Today, 295 entries of cherimoya, 5 of atemoya (*A. cherimola x A. Squamosa*) and 13 of other Annonaceae are included. The following characters are studied:

Fruit components
Epidermis type and resistance to penetration
Brix and acidity of the mesocarp
Seed index
Seed coat resistance
Seed size and colour
Leaf characteristics (lamina and petiole)
Stomatal density
Thickness of leaf lamina
Trichomes (epidermis, mesocarp and seeds)
Susceptibility to Mediterranean fruit fly
Flower components (petals, peduncle and stigmatic cone)
Flower density
Flowering season

Manipulation by Pruning of Growth and Cropping of Cherimoya (*Annona cherimola Mill.)*

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Pruning methods for cherimoya Fino de Jete

Modern pruning methods reduce cherimoya tree size from 4 to 2 meters height. Four pruning treatments were compared in a 4 years experiment:

- Traditional pruning allowing tree growth, with trees planted at 8 x 8 m square.
- Total pruning eliminating all previous year shoots.
- Semi total pruning , equal to total pruning but keeping a few widely scattered previous year shoots.
- Mean pruning, cutting the previous year shoots by approximately 2/3.

The last three treatments keep tree size constant and therefore trees were planted at 8 x 4 m . Yield per tree was highest with traditional pruning but its yield per hectare was lowest due to the lower number of trees.

Mean fruit size was highest with total pruning. A modified method of total pruning leaving short "thumbs" (about 5 cm long) appear to combine tree size control, high productivity and large fruit size. The experiment is proceeding along this line.

Out of season production of cherimoya

The total pruning previously described reduces natural fruit set to near zero. In midsummer approximately 1/6 of the new shoots are cut by half and a couple of leaves taken off. About a month later a new bloom starts in the naked buds. After hand pollination a crop is produced, out of season, in March - April. Not al cultivars are well adapted to this technique.

Micropropagation of cherimoya (*Annona cherimola* Mill.) cv. Fino de Jete

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Cherimoya (*Annona cherimola* Mill.) is a difficult-to-propagate species using traditional methods for vegetative propagation because of the low rooting capacity of adult cherimoya material. This problem has been solved by the development of clonal micropropagation protocols (Encina *et al*., 1994, Padilla, 1997) after nodal explants of juvenile and adult *Annona cherimola* cv 'Fino de Jete'. After sprouting and multiplication of axillary shoots on MS basal medium supplemented with different cytokinins (BAP and zeatin), a 95% rooting efficiency in juvenile material (Encina, 1992) was achieved in three steps:

1) pretreatment of *in vitro* shoots for 7 days on MS medium containing activated charcoal (0.1%);
2) root induction on MS medium supplemented with IBA (500 μM), sucrose (58.4 mM) and citric acid (200 mg l^{-1}) for 10 days (7 days in the dark followed by three days light); and
3) elongation of roots in half strength MS medium. The 70% acclimatization rate then recorded, has later been increased to 100% (Encina *et al*., 1999a). Using a similar method adult explants of cherimoya have been micropropagated althought rooting and acclimatization were about 30% less efficient (Padilla and Encina, in press).

The micropropagation protocol is being adapted to other selected cherimoya cultivars and rootstocks. A new system for aseptic establishment of explants is under study, i.e., the application of a modified micrografting protocol ("minigrafting") in cascade, increases the sprouting and shoot development in all cultivars assayed. Other complementary methods such as inoculation of micropropagated plantlets with arbuscular mycorrhizal fungi (*Glomus* spp.) improve vegetative growth and development of plantlets in the glasshouse and substantially reduce acclimatization time and increase plant recovery (Azcon -Aguilar *et al*., 1994a, 1994b, 1996).

References

Azcón-Aguilar, C., Encina, C.L., Azcon, R. and Barea, J.M. (1994a) Mycotrophy of *Annona cherimola* and the morphology of its mycorrhizae. *Mycorrhiza* 4, 161-168.

Azcón-Aguilar, C., Encina, C.L., Azcon, R. and Barea, J.M. (1994b) Effect of arbuscular mycorrhiza on the growth and development of micropropagated *Annona cherimola* plants. *Agricultural Science in Finland* 3, 281-287.

Azcón-Aguilar, C., Encina, C.L., Barceló-Muñoz, A., Herrero-Castaño, A. and Pliego-Alfaro, F. (1994) *In vitro* morphogenesis of juvenile *Annona cherimola* Mill. bud explants. *Journal of Horticultural Science* 69, 1053-1059.

Encina, C.L. (1992) Rooting method for cherimoya (*Annona cherimola* Mill.) cv. Fino de Jete, semihardwood cuttings, micropropagated by tissue culture. Patent nº 9200401. Spain. 12 pp.

Encina, C.L., Padilla, I.M.G., Cazorla, J.M. and Caro, E. (1999a) Tissue culture in cherimoya. *Acta Horticulturae* 497, 289-294.

Padilla, I.M.G. (1997) *Micropropagation of cherimoya (Annona cherimola Mill.) cv. Fino de Jete*. Unpublished PhD. thesis, University of Málaga, Malaga, 334 pp.

Padilla, I.G., Encina, C. L., Azcón, R. and Barea, J. M. (1996) Arbuscular mycorrhizal inoculation enhances plant growth and changes root system morphology in micropropagated *Annona cherimola* Mill. *Agronomie* 16, 647-652.

Regeneration and genetic transformation in cherimoya (*Annona cherimola* Mill.).

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Regeneration of multiple adventitious shoots from hypocotyl has been reported in most of the species of *Annona* studied: *A. squamosa* (Lemos and Blake, 1996a), *A. muricata* (Bejoy and Hariharan, 1992; Lemos and Blake, 1996b), *A. cherimola* x *A. squamosa* (Rasai et al., 1994) and *A. cherimola* (Jordan, 1988; Encina et al., 1999a).

Adventitious shoot development from leaf and internodal explants has been obtained in *A. muricata* (Lemos and Baker, 1998) and in atemoya (Rasai et al., 1994). In cherimoya protocols for adventitious organogenesis are still under development although regeneration at a low rate has already been obtained (Cazorla and Encina, unpublished results) from juvenile and adult material ('Fino de Jete'). Bud clusters from juvenile explants develop and root easily, but buds induced from adult explants require an elongation treatment to develop shoots.

Regeneration of cherimoya is focussed towards two main objectives: - the recovering of triploid genotypes (seedlessness) and – the developing of efficient protocols to develop transformation procedures using *Agrobacterium tumefaciens*.

Transformation of cherimoya is also under development, and is focussed to the introduction of genes (ACC synthase in antisense) in order to control fruit ripening which due to the short shelf -life of cherimoya fruits is the main commercial problem of this and other annonas limiting their expansion to remote markets. ACC synthase genes of cherimoya and atemoya have already been cloned and sequenced and the genes are available for transformation purposes (Encina et al., 2001a, b).

Future progress on *in vitro* tecnologies for rescue, selection and cloning of elite genotypes, development of efficient regeneration methods, modification on ploidy of these species, combined with results obtained using molecular genetic approaches will support advances with respect to genetic manipulation and improvement.

References

Bejoy, M. & Hariharan, M. (1992) *In vitro* plantlet differentiation in *Annona muricata*. *Plant Cell Tissue and Organ Culture* 31, 245-247.

Encina, C.L., Padilla, I.M.G., Cazorla, J.M. and Caro, E. (1999a) Tissue culture in cherimoya. *Acta Horticulturae* 497, 289-294.

Encina, C.L., Cavallaro, A.S. and Botella, J.R. (2001a) Ripening related ACC synthase from custard apple (*Annona cherimola* x *Annona squamosa*) GenBank# AF401757.

Encina, C.L., Diaz, J.A. and Botella, J.R. (2001b) Ripening related ACC synthase from cherimoya (*Annona cherimola* Mill.) GenBank# AF443280.(http://www.ncbi.nlm.nih.gov/GenBank/)

Jordán, M. (1988) Multiple shoot formation and rhizogenesis from cherimola (*Annona cherimola* L.) hypocotyls and petiole explants. *Gartenbauwissenschaft* 53, 234-237.

Jordán, M., Iturriaga, L. and Goreux, A. (1991). Promotion of *Annona cherimola in vitro* shoot morphogenesis as influenced by antoxidants. *Gartenbauwissenschaft* 56, 224-227.

Lemos, E.E.P. and Blake, J. (1996a) Micropropagation of juvenile and mature *Annona squamosa* L. *Plant Cell Tissue and Organ Culture* 46, 77-79.

Lemos, E.E.P. and Blake, J. (1996b) Micropropagation of juvenile and mature *Annona muricata* L. *Journal* *of Horticultural Science* 71, 395-403.

Lemos, E.E.P. and Baker, D.A. (1998) Shoot regeneration in response to carbon source on internodal explants of *Annona muricata* L. *Plant Growth Regulation* 25, 105-112.

Nair, S., Gupta, P.K., Shirgurkar, M.V. and Mascarenhas, A.F. (1984a) *In vitro* organogenesis from leaf explants of *Annona squamosa* Linn. *Plant Cell, Tissue and Organ Culture* 3, 29-40

Rasai, S., Kantharaj, A.S. and Dodd, W.A. (1994) The effect of growth regulators, source of explants and irradiance on *in vitro* regeneration of atemoya. *Australian Journal of Botany* 42, 33

Development of molecular markers for identification and breeding of cherimoya (*Annona cherimola* Mill.)

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Introduction

The cherimoya Germplasm Bank of the E.E. la Mayora maintains over 300 accessions obtained in diverse geographical areas, 295 of which are cherimoyas. An optimal management of the Bank requires a precise identification of the accessions as well as an evaluation of the diversity maintained. Traditionally, as in other plant species, cherimoya cultivars have been classified according to phenotypic traits that may vary among trees of the same cultivar depending on various factors such as location, year, etc. Consequently, a molecular characterization of the genotypes becomes necessary following similar procedures to those used with other fruit tree species (Wünsch and Hormaza, 2002). Very few works have been carried out in the field of identification and similarity studies in cherimoya using molecular markers. Some have included the use of isozymes (Ellstrand and Lee, 1987; Pascual et al., 1993; 1998) but those studies are limited by the number of loci. Some studies have used DNA-based markers, both RAPDs (Ronning et al., 1995) and AFLPs (Rahman et al., 1998) but only including a limited number of genotypes. Currently, microsatellites or SSRs are considered as the molecular markers of reference for genotype identification in plants due to their genetic (codominance and high degree of polymorphism revealed) and technical (reproducibility and easy automation) qualities. Microsatellites have been found in all the genomes analyzed and consist of tandemly repeated motifs of 1 to 6 bases flanked by conserved sequences that allow their amplification through PCR. They are present in both coding and noncoding regions and are characterized by a high degree of length polymorphism. Although microsatellites are a very interesting tool for different genetic applications, their major drawback is their isolation process that requires the development of a genomic library, the search and sequencing of clones with the repetitive sequences and the design of primers that amplify the microsatellite. Traditional methods of library screening (Rassmann et al., 1991) are only effective in species with a high frequency of microsatellites or when just a very small number of microsatellites is needed. Thus, the purpose of this work is to develop genomic libraries in cherimoya using a microsatellite enrichment procedure.

Materials and Methods

Two libraries were made from DNA of the cultivar "Fino de Jete" digested with *Pst*I and *Rsa*I following an enrichment procedure with (CT)n sequences (Billote et al., 1999). The selective hybridization process involves the biotinylation of the (CT)n oligonucleotide and the selection of the hybridized DNA by using streptavidin-coated magnetic beads. After selective hybridization, the captured fragments were recovered by PCR and cloned using the pGEM-T easy plasmid (Promega, Madison, USA) in *E. coli* ultracompetent cells (Epicurian coli© XL1 blue ultracompetent cells, Stratagene) following the manufacturer's recommendations. The recombinant clones were PCR amplified and transferred to a nylon membrane that was further hybridized with a (CT)15 probe labeled with digoxigenin (DIG oligonucleotide 3' -end labeling kit, Roche). Positive clones are then sequenced to identify the flanking regions that are used to design appropriate primer pairs. The microsatellites obtained are studied on a reduced group of 5 cherimoya cultivars by PCR amplification and high resolution agarose gel electrophoresis (Hormaza, 2002).

Results and discussion

We have developed two CT enriched genomic libraries from the cherimoya cultivar 'Fino de Jete' using the restriction enzymes *Pst*I and *Rsa*I. Although the work is in progress, the preliminary results obtained so far indicate that the procedure followed is appropriate to develop microsatellites in cherimoya. An example of the amplification obtained with one of the microsatellites obtained is shown in Fig. 1. The microsatellites obtained are validated on a reduced group of 5 cherimoya genotypes to select those that produce clear and repeatable amplification patterns. Subsequently, the selected microsatellites are used to screen the entire germplasm bank and they will also be tried in other species of Annonaceae. Our objective is to have by the end of 2003 a set of around 100 microsatellites that will be used in fingerprinting the accessions maintained in the cherimoya germplasm bank as well as to infer genetic similarity relationships among those accessions. Those results will be compared to phenotypic data to assess the relationships between both kinds of fingerprinting methods.

References

Billote, N., P.J.L. Lagoda, A-M. Risterucci, F.-C. Baurens (1999) Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. Fruits 54: 277-288.

Ellstrand, N.C., J.M. Lee (1987) Cultivar identification of cherimoya *(Annona cherimola* Mill.) using isozyme markers. Scientia Horticulturae 32: 25-31.

Hormaza, J.I. (2002) Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using Simple Sequence Repeats. Theoretical and Applied Genetics 104: 321-328.

Pascual, L., F. Perfectti, M. Gutierrez, A.M. Vargas (1993) Characterizing isozymes of Spanish cherimoya cultivars. HortScience 28: 845-847.

Perfectti, F., L. Pascual (1988) Characterization of cherimoya germplasm by isozyme markers. Fruit Varieties Journal 53: 53-62.

Rahman, M.S.M., T. Shimada, T. Yamamoto, J.Y. Yonemoto, M. Yoshida (1998) Genetical diversity of cherimoya cultivars revealed by Amplified Fragment Length Polymorphism (AFLP) analysis. Breeding Science 48: 5-10.

Rassmann, K. C. Schlötteres, D. Tautz (1991) Isolation of simple-sequence loci for use in polymerase chain reaction-based DNA fingerprinting. Electrophoresis 12: 113-118.

Ronning, C.M., R.J. Schnell, S. Gazit (1995) Using randomly amplified polymorphic DNA (RAPD) markers to identify *Annona* cultivars. Journal of the American Society for Horticultural Science 120: 726-729.

Wünsch, A., J.I. Hormaza (2002) Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. Euphytica 125(1): 59-67

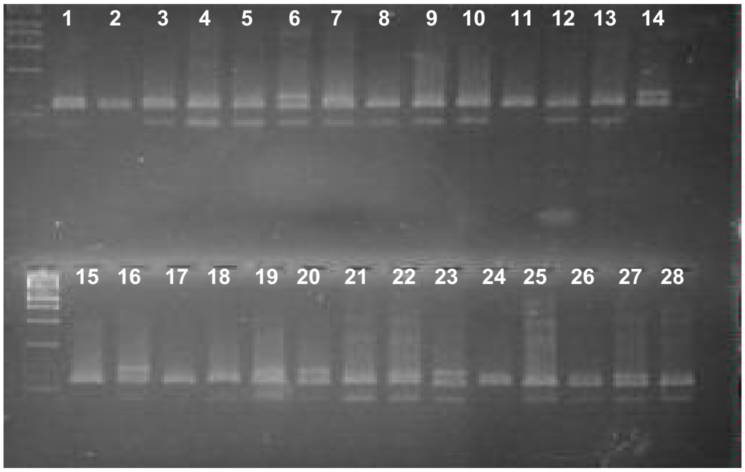

Figure. 1: Amplification of 28 cherimoya cultivars with microsatellite *lmch1*.

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The 12th Flora of Thailand meeting, 25 and 29th of November 2002 Bangkok.

Report of the coordinator for Annonaceae for the Flora of Thailand

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The Forest Herbarium (BKF), National Park, Wildlife and Plant Conservation Department, Bangkok Thailand organized the 12th Flora of Thailand meeting between 25 and 29th of November 2002 in Bangkok. There the coordinator presented a plan in which various national and international students will treat the family within the next three years. The final manuscript will be presented according to the schedule in the first half of 2006.
During a meeting with those colleagues who where interested to join the working group several people committed themselves to study certain genera and deliver manuscripts at the end of next year (see survey, next page). We would like to invite those colleagues who are willing to study one or several genera to contact the coordinator (Kessler@nhn.leidenuniv.nl).

During the meeting the wish was brought forward to organize a common field excursion in Thailand with the whole Annonaceae workgroup in 2004. Narong Koonkhuntod and the coordinator will explore possibilities.

The coordinator would like to thank the organizing committee, especially Dr. Kongkanda Chayamarit, for their hospitality and a their support of our working group.

Survey of genera to be treated in the scope of the Flora of Thailand

Name of genus	Annonaceae Nr. of species	treated by	revision exists
Alphonsea	4	Kessler	x
Anaxagorea	2	Narong Koonkhunthod	x
Cyathostemma	6	Utteridge	x
Meiogyne	2	Narong Koonkhunthod	x
Mezzettia	1	Chatchai Ngamriabsakul	x
Miliusa	9	Mols/Kessler	x
Mitrephora	7	Aruna	x
Monocarpia	1	Mols	x
Orophea	6	Kessler	x
Phaeanthus	1	Mols	x
Platymitra	1	Kessler	x
Pseuduvaria	5	Su	x
Sageraea	1	Chatchai Ngamriabsakul	x
Stelechocarpus	1	Chatchai Ngamriabsakul	x
Total nr. of species	47		

Annona	4	
Anomianthus	1	
Artabotrys	10	
Cananga	2	Phongsak Phonsema
Cleistopetalum	2	
Cyathocalyx	2	Wang
Dasoclema	1	
Dasymaschalon	7	Ada Ng
Dendrokingstonia	1	
Desmos	3	Ada Ng
Enicosanthum	3	Wichan Eiadthong
Fissistigma	5	
Friesodielsia	4	
Goniothalamus	22	Saunders
Marsypopetalum	1	Chatchai Ngernsaengsaruay
Melodorum	2	
Mitrella	1	
Neo-uvaria	2	Shih-Chung/Kessler
Polyalthia	23	Pasakorn Bunchalee ?
Popowia	1	
Trivalvaria	1	
Uvaria	9	Meade?
Xylopia	2	Johnson
Total nr. of species	109	

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