

Простоцветник, двуручник и другие самшитовые

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How everything started

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***Hapтанthus*, a New Dicotyledonous Genus from Honduras**

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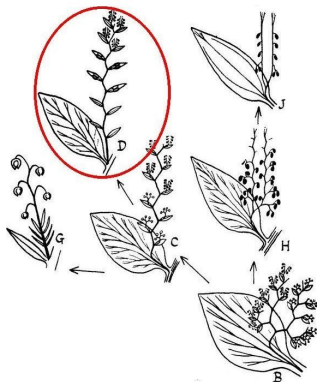
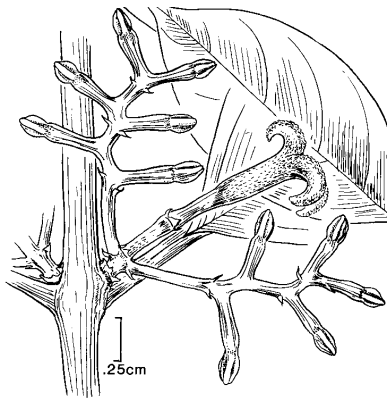
ABSTRACT. *Hapтанthus hazlettii* genus novum monotypicum is described. The plant is a dicotyledon with a combination of characters not found in any family. The inflorescences consist of a single central carpellate flower and two branches of 5–6 staminate flowers each. The flowers lack a perianth, merely being subtended by a minute bracteole. The staminate flowers are monandrous and the carpellate flowers have a tricarpellate, stipitate pistil with three large sessile stigmas and ovary with three parietal placentas, each with 8–15 ovules in two ranks. The plant is a completely glabrous shrub. The leaves are opposite, simple, entire and not glandular punctate. Stipules are absent and no stipular scars are evident.

Hapтанthus hazlettii Goldberg & Nelson, gen. et sp. nov. (figs. 1–3).—TYPE: Honduras, Atlantida, 5 km SE of Mataras, 8 Apr 1980, (fig. 1b, c); peduncle of staminate part of inflorescence 6–7 mm long, the internode between staminate flowers 1–3 mm, rarely 2 flowers may

Haptanthus hazlettii

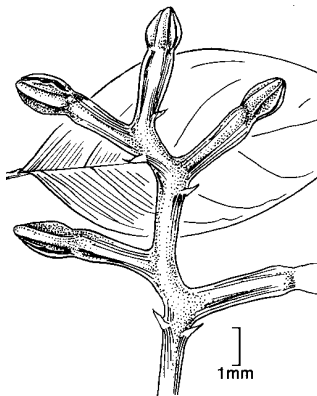
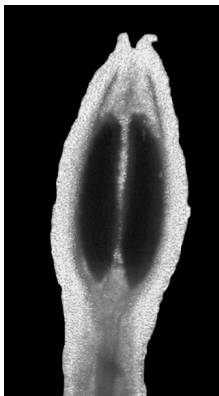
- ▶ One of the most rare plants in the world
- ▶ Discovered in herbarium collections made in 1980 in North Honduras
- ▶ Has unique and unusual reproductive structures which is hard to interpret
- ▶ Did not appear to be a member of any described family of angiosperms

Peculiar flower morphology



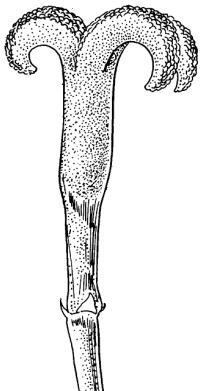
Female organs (pistils with 3 carpels?) are surrounded by branched clusters of male organs (stamens??). In all, reproductive structures superficially resemble some of R. Melville's (1962, 1963) diagrams of flower evolution.

Haptanthus reproductive organs: male



Male organs are most probably not single stamens, but complex structures originated from adnate sterile structures and 2 stamens (Doust & Stevens, 2005)

Haptanthus reproductive organs: female

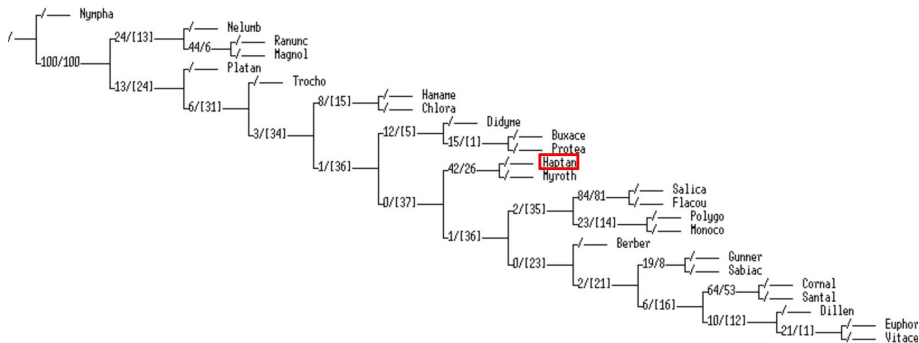


Female organs are single, elongated 3-carpellate pistils with 3 stigmas, and multiple parietal ovules

Making a family

- ▶ Attempts to determine plant with all known floras of Neotropics failed
- ▶ Two most widely used keys for description of angiosperm families—Hansen & Rahn and Thonner's do not give an answer
- ▶ Comparisons with somewhat similar families—Lacistemataceae, Flacourtiaceae, Buxaceae, Chloranthaceae, Euphorbiaceae also failed to recognize similarity; specialists working with these groups denied the putative membership
- ▶ As a result, C. Nelson in 2001 and A. Shipunov in 2003 recognized *Haptanthus* as a separate family, Haptanthaceae. Descriptions lack the information about fruits.

Morphological trees



Morphological methods also did not give a definite answer, support for all putative placements is low, not least of all that many useful characters remain unknown (TNT phylogenetic tree, data from Nandi et al., 1997).

Incertae sedis (placement unknown)?!

- ▶ Basal eudicots? (perhaps, Buxaceae)— Doust & Stevens, 2005
- ▶ Salicaceae—Euphorbiaceae? (Malpighiales)— Goldberg & Alden, 2005

Dicotyledonous Family of Incertae Position

Dicotyledonous Family of Incertae Position

1. HAPTANTHACEAE

C. Nelson 2002. (Isonym: Haptanthaceae Shipunov in Zhurn. Obshchei Biol. 64: 504, 2003, validated by a diagnosis in Latin). 1/1. Honduras (from 5 km south-east of Mataras, Atlantida).

Evergreen glabrous tree. Vessels with scalariform perforations or scalariform and reticulate; scalariform

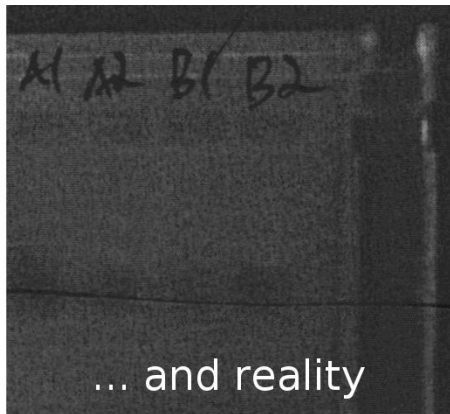
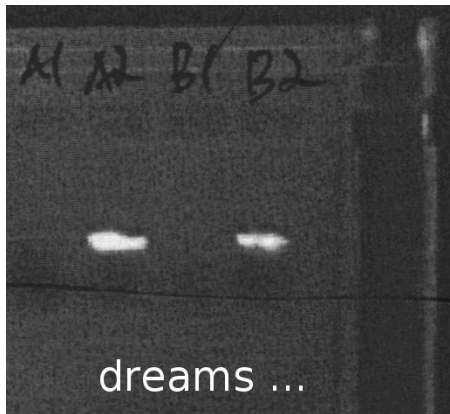
- ▶ Armen Takhtajan (2009) regarded *Hapтанthus* as an only unplaced, *incertae sedis* family among angiosperms.

Only two herbarium samples



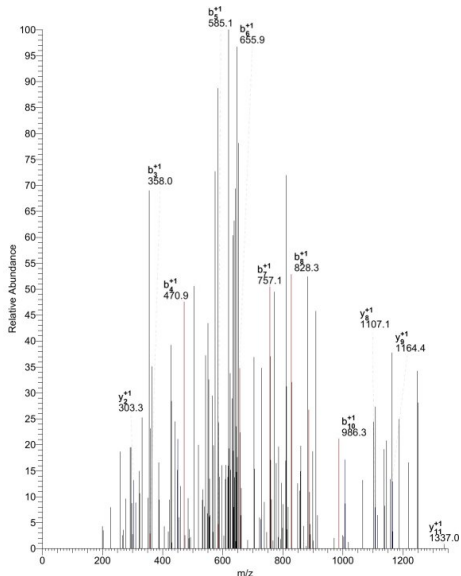
- ▶ One herbarium sheet is kept in Missouri Botanical Garden, the second—in Lancetilla Botanical Garden (Tela, Honduras)
- ▶ All attempts to extract DNA (and even proteins) failed

DNA detection failed



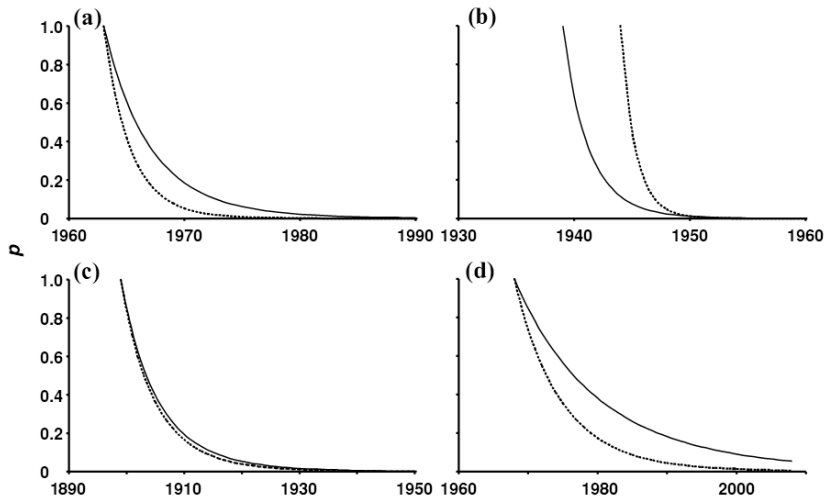
Proteins detection failed

#403-403 RT:14.40-14.40 NL: 1.85E6



To check the method, we used 50-year-old herbarium specimen of *Alnus* to extract RuBisCo and were able to obtain more than 42% protein sequence coverage by liquid chromatography/tandem mass spectrometry analysis.

Extinct or alive?



Statistical estimation of the time frame between the date of last sighting and predicted date of extinction for New Zealand birds (Roberts et al., 2009) usually gives several dozens years. This was our only hope, because five (!) attempts to find the plant failed.

Tela, Atlantida province, Honduras



Taking this into account, in April 2010 we organized small expedition to North Honduras.

Lacentilla Botanical Garden



Now we have the better map



No forest anymore...



... but wildlife is around



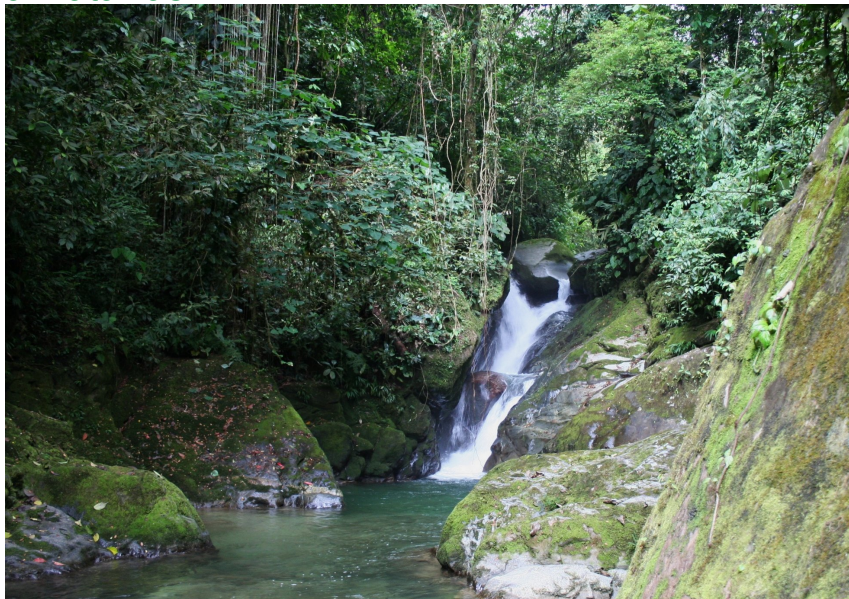
Forest is better towards mountains and on slopes



The main camp



Rio Matarras



Basilisk lizard



Search strategy



The main strategy was to search along borders of tree cuts/pastures/plantations. Most of flowering small trees are concentrated there

Finding



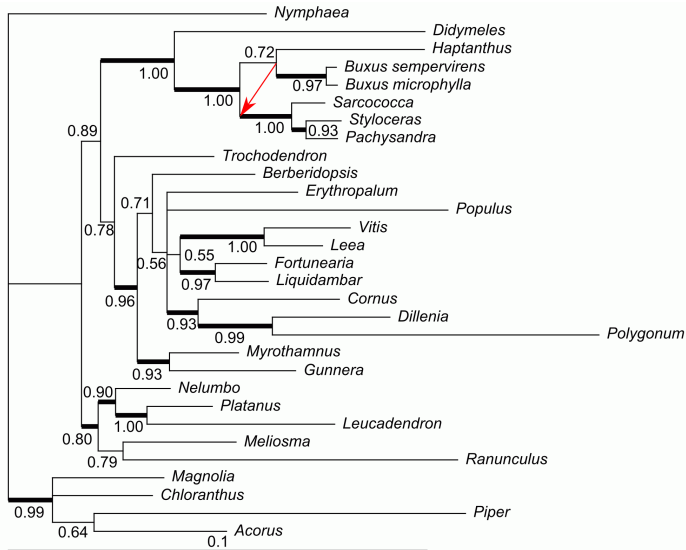
Finally, from the top of the hill (≈ 400 m altitude) we saw with binoculars unusual small tree, and that was *Haptanthus*!

Haptanthus is alive!



(April 23rd)

DNA



Molecular phylogeny: 100% Buxaceae, boxwood family

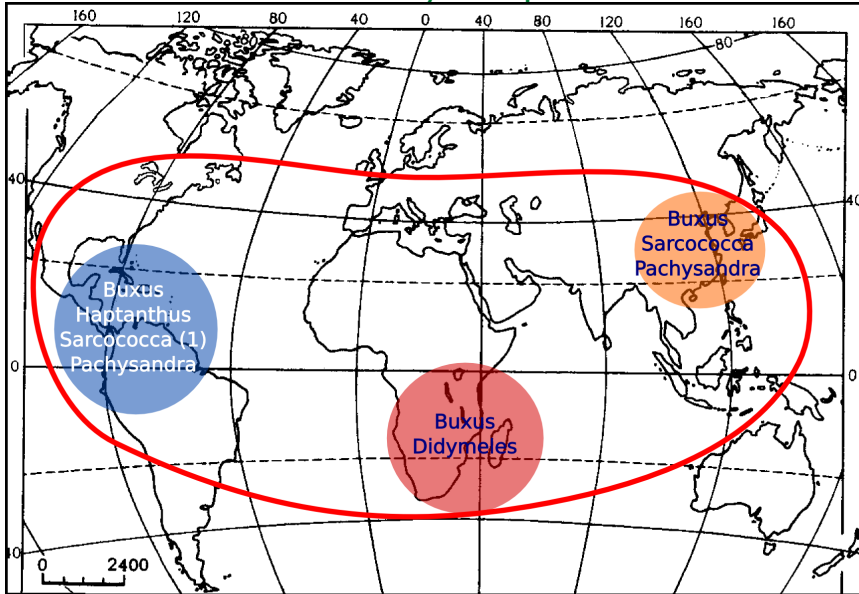
[From Shipunov & Shipunova, 2011]

Genera and biogeography

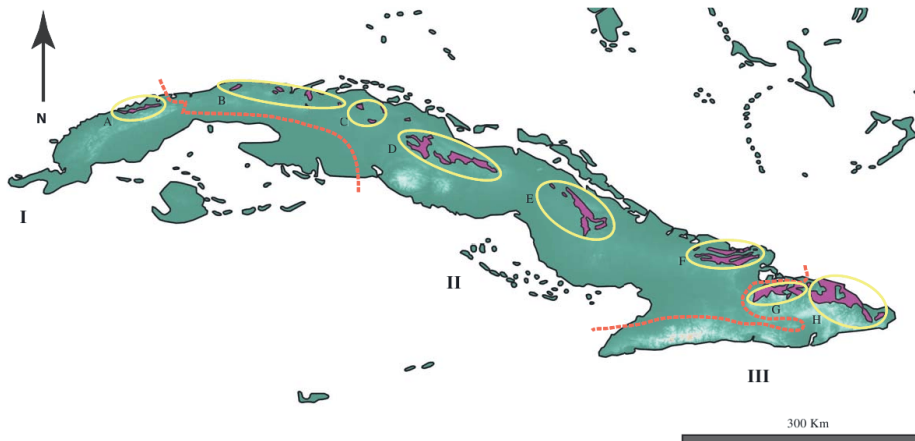
- ▶ Six genera comprise about 138 species
- ▶ Distributed worldwide but mainly associated with Tropical America, East and South Africa and Madagascar, and East Asia (Köhler, 2007)
- ▶ The largest are well-known (mostly from widely cultivated *Buxus sempervirens*) tree-like or shrubby boxwoods, *Buxus*

Pachysandra
 Sarcococca
Buxus
 Styloceras Haptanthus
 Didymeles

Distribution and biodiversity hotspots of Buxaceae



Cuba is the boxwood biodiversity hotspot



37 species, 35 are endemic!
(From Gutierrez, 2014)

Boxwood, *Buxus* and its inflorescences



Styloceras



- ▶ Few species of woody *Styloceras* grows in South America
- ▶ All are little known to science

Sarcococca and *Pachysandra*

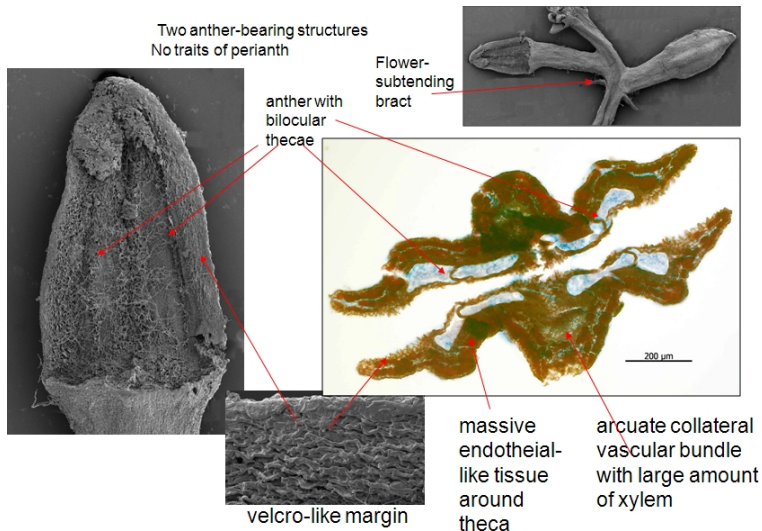
- ▶ Small genera of low shrubs (*Sarcococca*) or even rhizomatous herbs (*Pachysandra*)
- ▶ Some of them are also popular in gardens
- ▶ *Pachysandra* has remarkable distribution: East Asia (2 species) and USA East Coast (1 species)

Pachysandra and *Sarcococca*



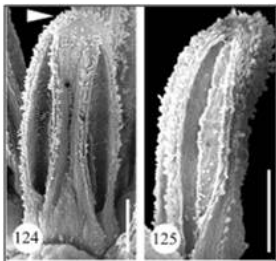
Morphology of *Haptanthus* fructifications

Male organs of *Haptanthus* are most weird

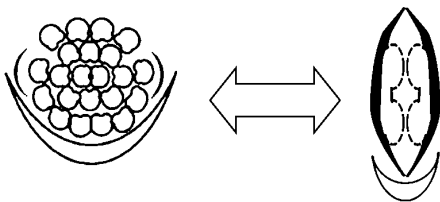


“Stamens” are not stamens!

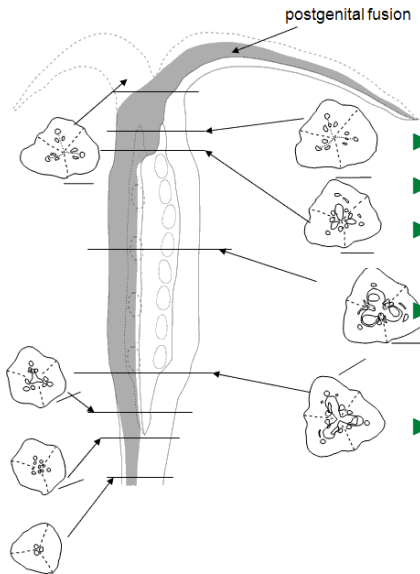
Styloceras vs. *Haptanthus*



[Photo from M. von Balthazar and P. Endress (2002a, b)]



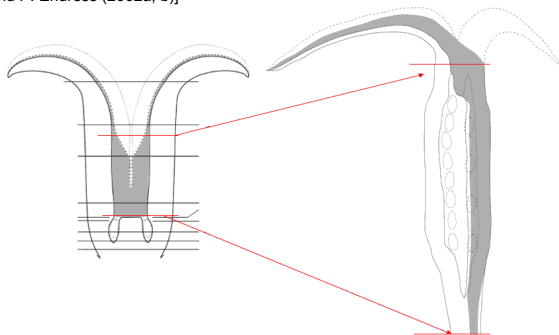
Female flower



- ▶ Parietal placentation
- ▶ 8–15 ovules per carpel
- ▶ No synascidiate zone (only symplicate zone)
- ▶ Two rows of ovules in each carpel (cf. with two ovules per carpel in other Buxaceae)
- ▶ Vascular anatomy is also similar to other Buxaceae

Sarcococca vs. Haptanthus

[Picture of *Sarcococca*
from M. von Balthazar and P. Endress (2002a, b)]



- ▶ *Sarcococca* has the most prominent symplicate zone (but two carpels only)
- ▶ Parietal placentation is maybe a result of *symplicate zone elongation*—a possible way to increase the seed number per fruit.

Conservation



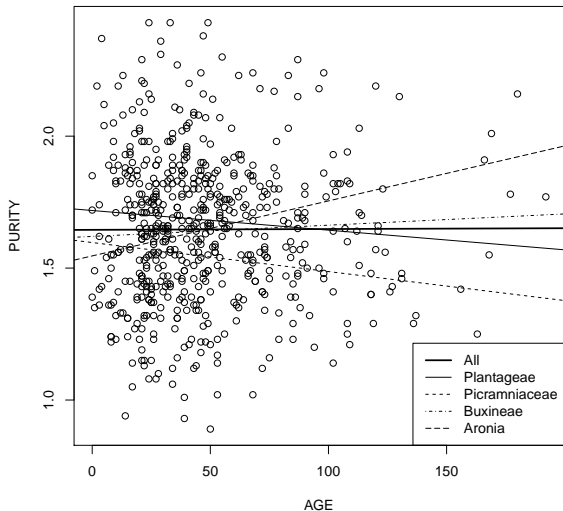
We found only one tree, but mared the point with GPS so two months later almost twenty trees have been found, and the one branch has been rooted and planted in Lancetilla Botanical Garden

Fruit

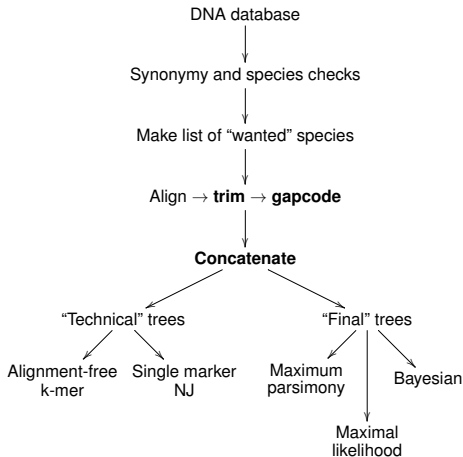


In 2014, they finally discovered the fruit of *Haptanthus*!

DNA purity suffers only a little from the age of sample

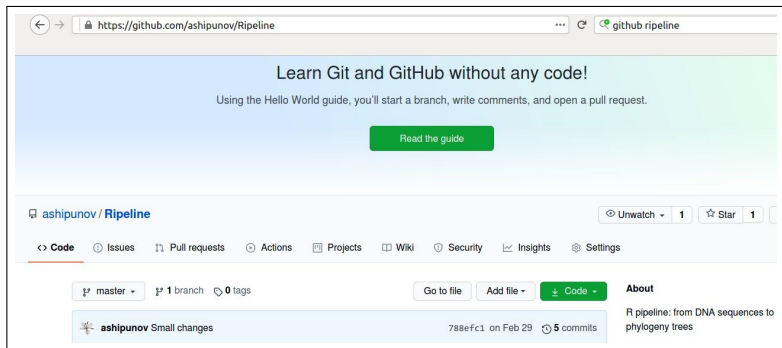


Ripeline



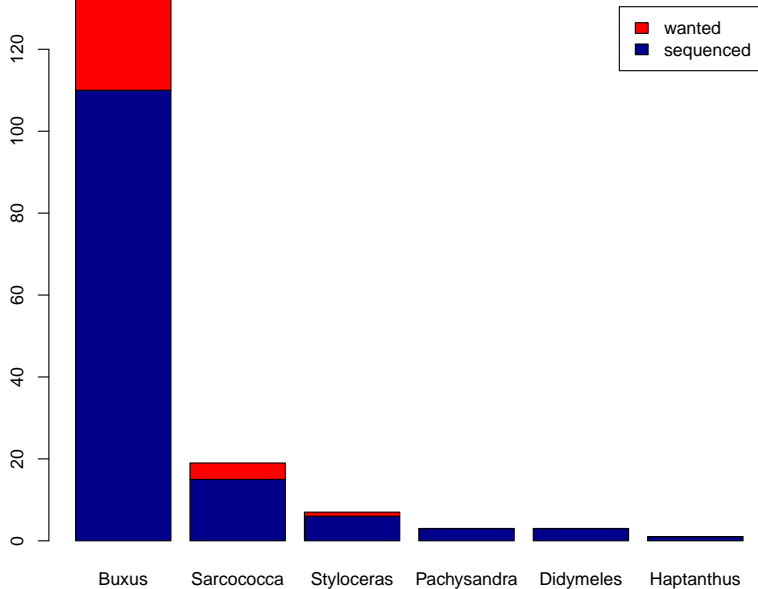
That amount of work is possible to manage with **Ripeline**, semi-automatic R-based workflow which is capable to deal with thousands of sequences.

Ripeline oh Github

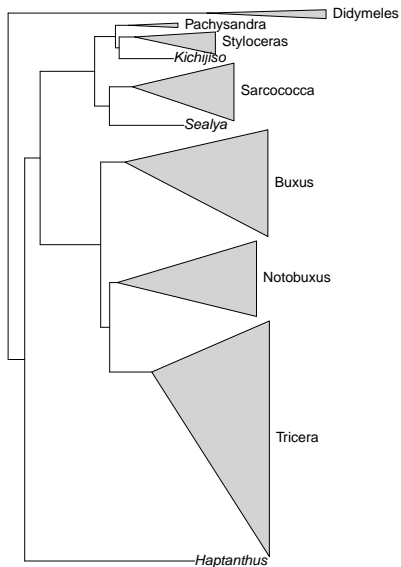


Ripeline is the fully customizable set of R scripts, it is free software available on Github.

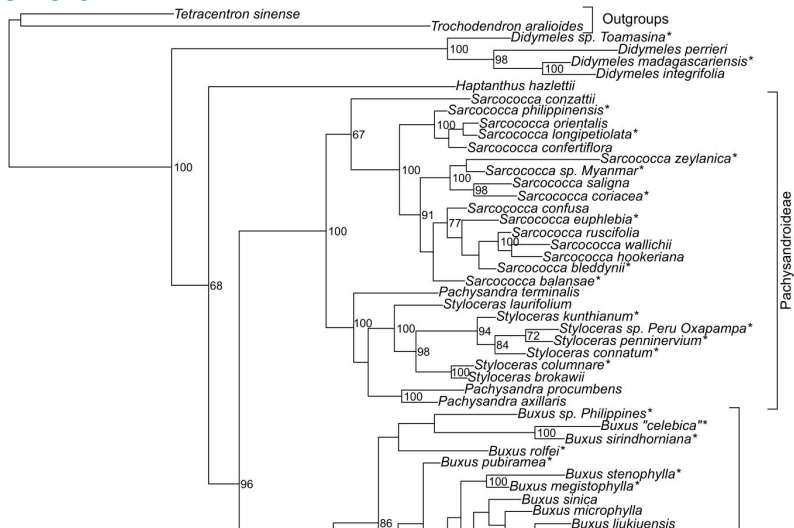
Coverage



ツゲ科: *Buxaceae sensu lato*

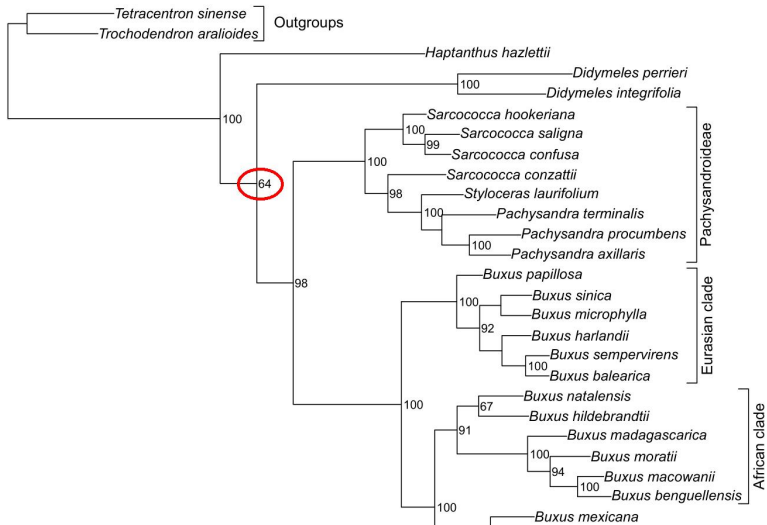


We increased the amount of molecular data at least two-fold



Stars* designate species sequenced for the first time

With more markers, support increases dramatically



... however, we still unable to resolve *Haptanthus*–*Didymeles* node

Didymeles toamasinae nom.prop., new species from Madagascar



Kichijiso terminalis nom.prop. and *Sealya konzattii*
nom.prop.



Pachysandra (“Kichijiso”) *terminalis*



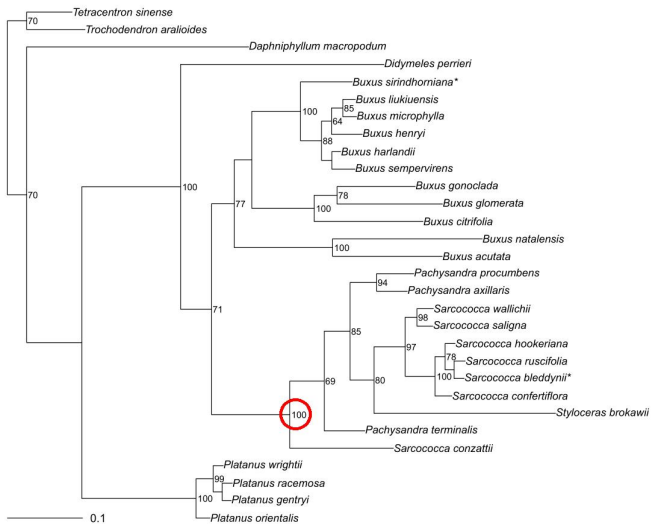
Inflorescences terminal; gynoecium 2-carpellate; fruits white, drupaceous.

Sarcococca (“*Sealya*”) *conzattii*



Female flowers terminal on inflorescences; gynoecium 2-carpellate; fruit with dry mesocarp, white. Described first as *Buxus*, the only representative of *Sarcococca* in New World (southern Mexico and Guatemala).

“Full ITS” tree



More data typically gives more support for both “*Sealya*” and “*Kichijiso*”.

Personal acknowledgements

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