Virola dominicana sp. nov., (Myristicaceae) from Dominican amber

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Abstract: The Myristicaceae is a member of the early diverging angiosperm order Magnoliales, however the family is poorly represented by fossil collections. We describe *Virola dominicana* sp. nov. (Myristicaceae), the first record of fossilized Myristicaceae flowers, from mid-Tertiary (45-15 mya) Dominican amber**.** The description is based on 24 male flowers in 17 pieces of amber, thus providing some indication of intra-specific variation, including a 2-tepaled flower. Diagnostic characters of the new species are the long simple or few-branched trichomes on the perianth margins, the small pollen grains and a short staminal column. There are no endemic members of the Myristicaceae in Hispaniola today and it is speculated that *V. dominicana* disappeared from the region during the Pliocene-Pleistocene cooling events. These fossils provide observations of possible pollinators of *Virola* and establishes the presence of Myristicaceae in the Western Hemisphere during the mid-Tertiary.

Keywords: *Virola dominicana*, Myristicaceae, Dominican fossil Amber, paleobotany

Introduction

Amber, the fossilized resin of woody plants, may contain a great wealth of ancient organisms and is one of the best media for preserving delicate structures like flowers. Not only is the perianth often preserved, but also details of trichomes and even complete pollen cells. Flowers up to 100 million years old have been preserved in amber, although these cannot be assigned to extant genera (Poinar and Chambers 2005; Chambers et al. 2010). Flowers found within Dominican amber have been used to identify members of the genera *Persea* (Lauraceae)(Chambers et al. 2011a), *Trichilia* (Meliaceae) (Chambers et al. 2011b), and *Trochanthera* (Balanophoraceae or Moraceae) (Poinar et al. 2008).

The Myristicaceae comprises an assemblage of canopy to subcanopy trees of some 21 genera and about 500 species distributed in tropical and subtropical regions of Central and South America, Asia, Africa and Madagascar (Doyle et al. 2004; Smith 1937). Despite the family's phylogenetic placement within the early diverging magnoliid grade of angiosperms (Soltis et al. 2011), the Myristicaceae are poorly represented by fossil collections. Fossilized wood (Boureau 1950), leaves (Wolfe 1977), pollen (Frederiksen 1973; Jan du Chene et al. 1978), fruits and seeds (Berry 1929) have been described as belonging to the Myristicaceae, however few can be unambiguously assigned to the family.

Six genera of Myristicaceae are endemic to the Neotropics. One of these is *Virola*, which is represented by some 60 species restricted to lowland and cloud rainforests of Central and South America. *Virola* is differentiated from other Neotropical genera of Myristicaceae by possessing profuse red latex (rarely green-yellow) and dendritic or stalked-sessile stellate hairs on the surfaces of their leaves, flowers and fruits. The flowers of these dioecious trees are very small (1-4mm in diameter) and are composed of 3-4 tepals (petals) that are often covered in a dense dendritic or stellate pubescence. Extant species of *Virola* can only be identified by numerous vegetative and reproductive characters as there exists a great deal of overlap among species for any one character.

The objectives of this study are to 1) describe the staminate flowers of *Virola dominicana*, the only known fossil flowers of the family Myristicaceae, and 2) quantify the floral variation that occurred within *Virola* in Hispaniola (modern day Haiti and Dominican Republic) during the mid-Tertiary.

Materials and Methods

The Dominican amber specimens were obtained from mines in the Cordillera Septentrional, between Puerto Plata and Santiago, of the Dominican Republic. Dating of Dominican amber is equivocal with the latest proposed age of 20-15 mya based on foraminifera (Iturralde-Vinent and MacPhee 1996) and the earliest as 45-30 mya based on coccoliths (Cêpek in Schlee 1990). In addition, Dominican amber is secondarily deposited in sedimentary rocks, which makes a definite age determination difficult (Poinar and Mastalerz 2000). Dominican amber was produced by the leguminous tree, *Hymenaea protera* Poinar (1991) and a re-construction of the Dominican amber forest based on amber fossils indicated that the environment was similar to that of a present day tropical moist forest (Poinar and Poinar 1999).

Observations, drawings, and photographs were made with a Nikon SMZ-10 R stereoscopic microscope and Nikon Optiphot compound microscope with magnifications up to 600X. In some instances, Helicon Focus Pro X64 was used to stack photos for better clarity and depth of field.

Results

Seventeen pieces of Dominican amber, containing a total of 24 male and no female *Virola* flowers, were examined during the present study. Not all characters were evident on every flower since some had been partly polished away during preparation of the amber or partially eaten by herbivores or slightly obstructed from view by other inclusions. The number of specimens examined for each numerical value is provided.

Description

Myristicaceae

Virola Aublet, 1775

Type Species: *Virola dominicana* Poinar and Steeves, sp. nov. (Figs. 1-4)

Specific Description: Flowers brown to orange-brown; perianth 3 (2) -lobed to middle or beyond; lobes thick, rounded to acute at apex, spreading at anthesis; stamens 3, apparently opposite tepal lobes where visible in two specimens (supplementary Figs. S2+S3), filaments connate in a column; anthers 2-celled, extrose, dehiscing longitudinally. Length of the complete flower 3.4 (2.1-5.0) mm (N= 16); length of perianth lobes 1.3 (0.7-3.3) mm (N= 24); greatest width of perianth lobes 0.9 (0.5-1.5) mm (N= 23); length of fused portion of perianth 0.9 (0.5- 1.5) mm (N= 19); pedicel length 1.4 (0.7-2.0) mm (N= 17); length of stamenal column 0.5 (0.3-0.8) mm (N= 10); length stamen stalk 0.2 (0.1-0.3) mm (N= 6).

Exterior surface of perianth covered with short, stubby, simple or branched trichomes (supplementary Figure S1); lobe margins with long, thick single to multiple- celled trichomes ranging from 0.1 to 0.3 mm in length; pollen apparently monosulcate, boat-shaped, ranging from 23- 27 µm in diameter (Fig. 2F). Pistillate flower unknown.

Types: Holotype deposited in the Poinar amber collection (accession # Sd-9-30A) and Paratypes (Sd-9-30B through Sd-9-30R) maintained at Oregon State University, Corvallis, Oregon.

Type locality: Amber mine in the northern mountain ranges (Cordillera Septentrional) of the Dominican Republic.

Etymology: The specific name is based on the country of origin of the fossil.

Discussion

Despite their early origination in angiosperm evolution and their current pantropical distribution, the fossil record of the Myristicaceae is rather meager and no fossil flowers have been described prior to this study. Eocene fossil seeds and fruit casts from Texas were described as *Myristica catahoulensis* Berry (Berry 1929; Chesters et al. 1967), however Doyle et al (2008) postulate that these represent molds of an endocarp of Mastixiaceae (Cornales). Leaf fragments from the Eocene of Borneo were described as *Myristicophyllum minus* Geyler (Andrews 1970). Wood described as *Myristicoxylon princeps* E. Boureau from Oligocene-Miocene deposits in the Sahara was also attributed to the nutmeg family (Boureau 1950; Collinson et al. 1993). In addition, Miocene (~23-5 mya) and Eocene (~56-34 mya) fruits from Germany and England were assigned to the Myristicaceae and described as *Myristicacarpum miocaenicum* Gregor and *M. chandlerae* Doyle, Manchester and Sauquet, respectively (Collinson et al. 1993; Doyle et al. 2008). Myristicaceous pollen from the Eocene of Nigeria was described as *Echimonocolpites major* du Chene (Collinson et al. 1993).

A seed described as *Virola tertiaria* Berry from the Oligocene of Peru, is the only previous putative fossil of the genus *Virola* (Berry, 1929), however a transversely broken specimen does not possess tegminal ruminations, which would be indicative of *Virola* (Doyle et al. 2008). A flower of *Virola* in Dominican amber was previously depicted, but not described (Poinar and Poinar, 1999).

The male flowers described in this study are the first confirmed fossil flowers of the family Myristicaceae and represent one of few fossil collections distinctly of Myristicaceae origin. *Virola dominicana* is known only from these 24 flowers entrapped in 17 pieces of mid-Tertiary Dominican amber. The spreading petal lobes of *V. dominicana* resemble the modern day species of *V. surinamensis, V. multinervia, V. duckei and V. flexuosa*. However, these extant species have mostly stellate trichomes on the lobes (Smith, 1937; Croat 1978), as do other members of the genus (Sabatier, 1997), whereas the lobes of *V. dominicana* appear to possess simple or few-branched trichomes. The stamen column (anther length) of *V. dominicana* (0.3-0.8 mm) is considerably shorter than most extant *Virola* species (0.6-1.9 mm as measured in 8 extant *Virola* species [Smith, 1937; Steeves, 2011]). The shape and size range of the pollen of *V. dominicana* (23-27 µm) falls within the range of members of the genus (22-35 µm)(Walker and Walker, 1979). The two-petaled (or two-tepaled) flower (Figs. 2A and 2B) may represent a developmental anomaly as flowers comprised of two petal lobes are rarely observed in extant *Virola* (Steeves, personal observations). We may have found 24 male *Virola* flowers and no female flowers as extant species have staminate flowers that are easily deciduous owing to their thinner and longer pedicels compared to thick and stout pistillate flowers that rarely fall to the ground (Steeves, personal observation).

A molecular study estimated the crown group of the Myristicaceae to have a Miocene origin based on a molecular clock (Doyle et al. 2004), which is not incongruous with the estimated age of the present fossils. However, this relatively recent molecular age estimate is surprising given the pantropical distribution and relatively poor oceanic dispersal potential of the Myristicaceae and the earlier estimates of crown group divergence (~100-120 mya) of closely related families such as the Annonaceae (Doyle et al. 2004). This relatively young age estimate for the Myristicaceae could be an artifact of their poor fossil representation and/or a result of a slower rate of molecular evolution compared to closely related families, leading to a mis-calibration in the molecular clock estimate. Both the former and latter explanations are plausible given the paucity of fossil evidence of the family and the fact that molecular investigations employing some of the most variable nuclear and chloroplast DNA markers have found low levels of molecular divergence among genera and even species of Myristicaceae (Sauquet et al. 2003; Doyle et al. 2004, Steeves 2011). The fossil flowers described in this study place *Virola* in the neotropical region sometime between 45-15 mya depending on age estimates for Dominican amber (Iturralde-Vinent and MacPhee, 1996; Schlee 1990).

Extant members of *Virola* are of significant ecological and ethnobotanical importance in the neotropics. *Virola* spp. are considered one of the 5-10 most abundant fruit producing tree genera in South American (Pitman et al. 2001; Pitman et al. 2002). The bright orange-red, fat-rich aril fruits are highly nutritious and are highly sought after by birds and spider monkeys (*Ateles paniscus*).

Pollinators of *Virola* have been little studied. Beetles and thrips have been observed visiting flowers (Steeves, 2011), however no representatives of these groups occurred among the various insects found together with *Virola* flowers in the examined Dominican amber samples. Planthoppers occurred with *Virola* flowers in several amber pieces (Fig. 3) and may have been feeding on the perianth, possibly obtaining glandular secretions in the flower and/or feeding on the plants sap. Both worker and alate ants were associated with several *Virola* flowers in the amber samples. One of the identifiable ant species present in the amber along with *V. dominicana* is a worker *Cephalotus squamosus* (Hymenoptera:Formicidae) (Fig. 4). These ants are polyphagous but do include pollen in their diet and take pollen back to the nest for larvae and nestmates. Ants visiting male flowers could transfer pollen to female flowers, especially if the latter have extrafloral nectaries, which *Cephalotes* ants are known to visit (de Andrade and Baroni Urbani, 1999). Although extant *Virola spp.* are not known to possess extrafloral nectaries, ants have been observed on the male flowers of extant *Virola calophylla* (Steeves, 2011) and could conceivably transfer the pollen to nearby female trees. The small and rather inconspicuous flowers of *Virola spp.* are unlikely to be predominantly visually attractive to pollinators. The flowers do, however, emit pungent odours (similar in smell to lilacs) that appear to attract insects even after male flowers have fallen to the ground (Steeves, personal observation). It is possible that the insects observed in the inclusions inspected for this study represent individuals who were haphazardly trapped in the resin, but their close proximity to the flowers (particularly *Cephalotes squamosus*, Fig. 4) may indicate that the attraction of the fallen *Virola dominicana* flowers is what led to their entrapment.

The Dominican amber forest was categorized as a tropical moist forest unlike any other found in the World today. During the Pliocene-Pleistocene global cooling period, many of the stentopic biota of the forest, such as stingless and orchid bees, *Mastotermes* termites and various plants, including *Hymenaea protera* that produced the amber, were trapped by the island's isolation. Refugia were apparently rare or inadequate for many life forms (Poinar and Poinar, 1999). It is likely that *V. dominicana* disappeared from Hispaniola during this cooling period. Although there are no native members of Myristicaceae in Hispaniola today (Liogier 1983), *Virola surinamensis* is native to nearby Guadeloupe southward to Grenada in the Lesser Antilles (Smith 1937). The mainland distribution of contemporary *Virola* taxa encompasses lowland and montane rain forest habitats (<1500m elevation) from Guatemala to the southern and eastern extent of the Amazon Basin and the western coast of Ecuador (Smith 1937).

This study describes the first fossil flowers of the Myristicaceae, *Virola dominicana* Poinar and Steeves preserved in mid-Tertiary Dominican amber. The 24 male flowers preserved in amber provide a glimpse into the reproductive biology of a genus whose contemporary pollinators are not well established to date. The placement of *Virola* in the Neotropical region during the mid-Tertiary will undoubtedly help future studies of the biogeography and evolutionary biology of these ecological and ethnobotanically significant trees.

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Figures

Fig. 1. *Virola dominicana* sp. nov. holotype showing stubby trichomes on the lobe blades and long trichomes on the lobe margins. Note also white pollen grains within the flower diameter. Bar = 800 µm.

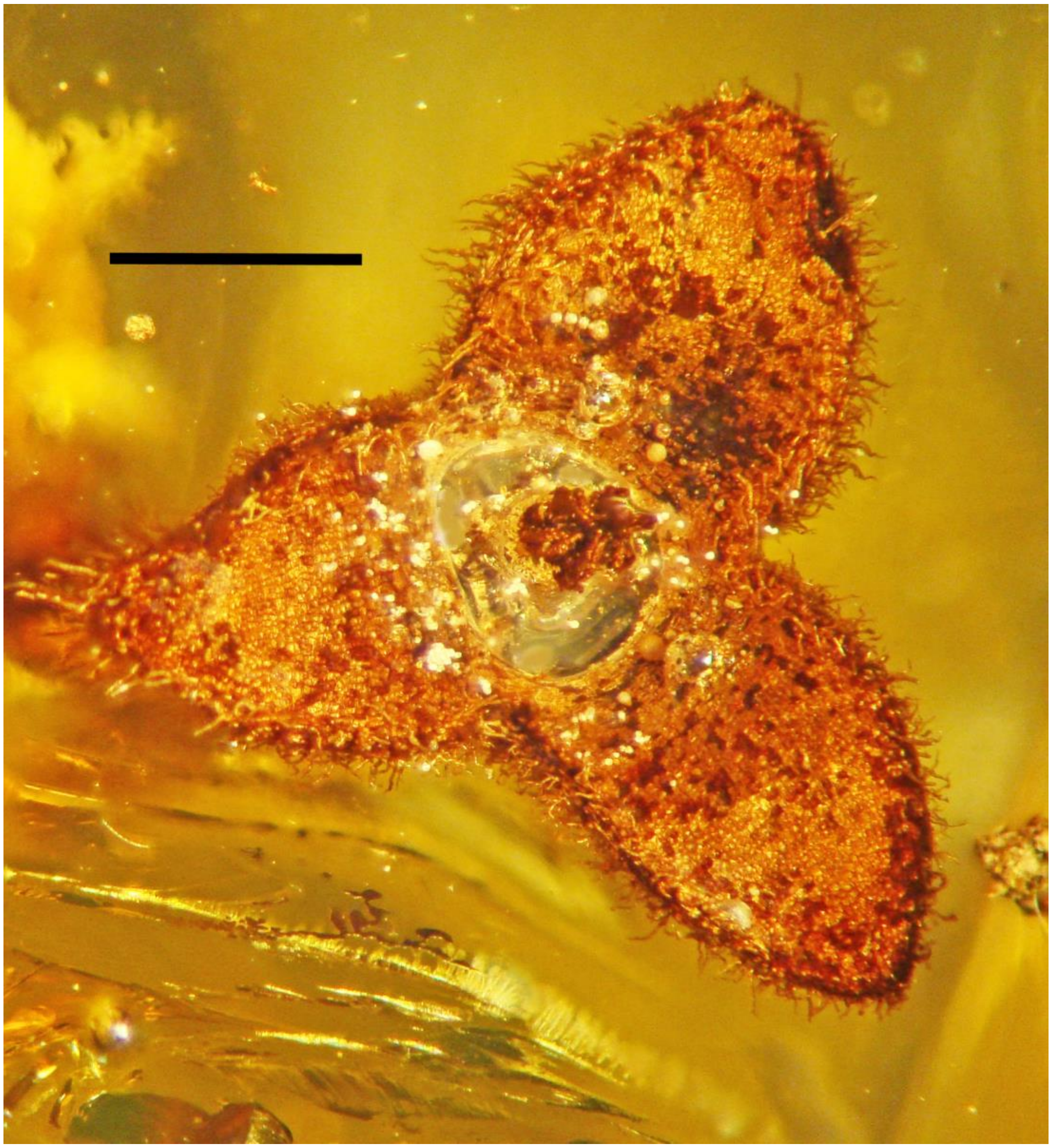

Fig. 2. Male flowers, indument and pollen of *Virola dominicana*. (A) Lateral view of paratype of *Virola dominicana* in Dominican amber. Bar = 850 µm. (B) Frontal view of two-lobed flower of *Virola dominicana* in Dominican amber. Bar = 480 µm. (C) Long, straight trichomes on the margin of a perianth lobe of the Holotype of *Virola dominicana* in Dominican amber. Bar = 105 µm. (D) Pollen grains of *Virola dominicana* in Dominican amber. Note upper boat-shaped grain. Bar = 23 µm. (E) Lateral view of two-lobed flower of *Virola dominicana* in Dominican amber. Bar = 28 µm. (F) Detail of pollen grains of the Holotype of *Virola dominicana* in Dominican amber. Bar = 22 µm.

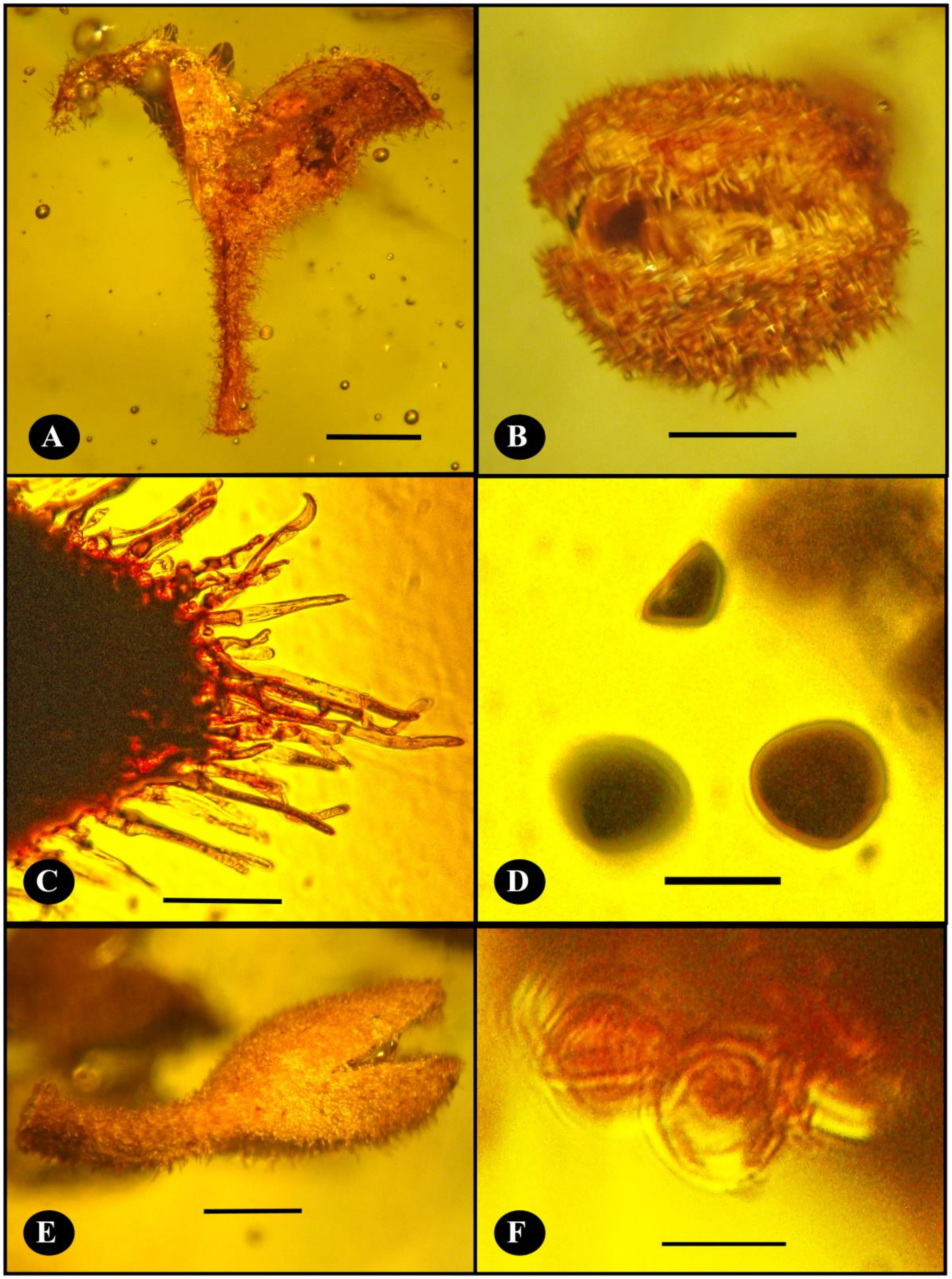
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Fig. 3. A planthopper (Hemiptera: Fulgoroidea) adjacent to a paratype flower of *Virola dominicana* in Dominican amber. Bar = 490 μm.

Fig. 4. A worker *Cephalotes squamosus* (Hymenoptera: Formicidae) with its head in an open paratype flower of *Virola dominicana* in Dominican amber. Bar = 1.4 mm.

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