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# Leaf essential oils and volatiles, histochemistry and micromorphology of *Neomitranthes obscura* (DC.) N. Silveira (Myrtaceae) growing in sandy coastal plains of Rio de Janeiro

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ABSTRACT

We evaluated the chemical composition of leaf essential oils and volatiles in *Neomitranthes obscura* from different sandy coastal plains in Rio de Janeiro State. These sites present different environmental conditions. Leaf essential oils and volatiles were characterized by GC/MS after simultaneous distillation-extraction (SDE) and hydrodistillation (HD). All samples were rich in sesquiterpenes, mainly those from cadinene, germacrene and caryophyllene cyclization pathways, including (*E*)-caryophyllene, α-copaene, β-selinene, α-cadinene, selina-3,7 (11)-diene, germacrene B and caryophyllene oxide. Chemical profiles of *N. obscura* from different *restingas* presented high variation in the relative amount of some compounds. Fewer compounds were produced in a semi-arid climate (Massambaba *restinga*). Comparing samples collected during different seasons in Marambaia *restinga*, (*E*)-caryophyllene presented highly variable concentrations. The highest concentration was observed in August, which is the month of least rainfall. In contrast, germacrene B presented the lowest concentration in August. Leaf secretory structures were described using light and scanning microscopy and histochemical tests. The main sites of lipophilic and terpenoid accumulation are located in the secretory cavities of mesophyll. Our data indicate that the production of essential oils and volatiles in *N. obscura* is influenced by both plant developmental stage and environmental conditions.

## 1. Introduction

*Neomitranthes* D. Legrand (Myrtaceae) is a native plant genus endemic to the Brazilian Atlantic Rainforest Domain. It consists of sixteen identified species and numerous synonyms, indicating a complex taxonomy of this group for which few studies are available in the literature (Sobral et al., 2016). *Neomitranthes obscura* (DC.) N. Silveira (Myrtaceae) is abundant and widely distributed in the sandy coastal plains (*restingas*) of Rio de Janeiro State in open shrubland not subject to flooding. This species is a large evergreen shrub, or small tree, reaching heights from 1.5 to 5 m (Fig. 1a–c). Insect galls are commonly found in lateral and apical buds of *N. obscura*, making it easy to identify this species since galls, in particular those of *Neomitranthella robusta* (Cecidomyiidae, Diptera), have obvious features (Maia, 1995) (Fig. 1f).

In Brazil, ethnobotanical information indicates that *N. obscura* is known as “cambuí-de-cachorro”, “pitanga-de-cachorro” and “bapuana” and that it is used by local populations as food and firewood (Fonseca-Kruel and Peixoto, 2004, Fig. 1c–e). It is a fruit plant with strongly aromatic and fleshy leaves and sweet and swollen flower petals commonly used as food for birds (Gomes et al., 2010). Recent studies have shown the presence of anthocyanin in fruit peels (Gouvêa et al., 2015), conferring different colors of pericarp, such as black and yellow/orange (Souza and Morim, 2008; Amaral et al., 2013). In Rio de Janeiro State, fruits with green, black and yellow pericarp in the same individual were found in the Massambaba *restinga*, most likely indicating different developmental stages (Fig. 1c,e). Similar to other Myrtaceae species,

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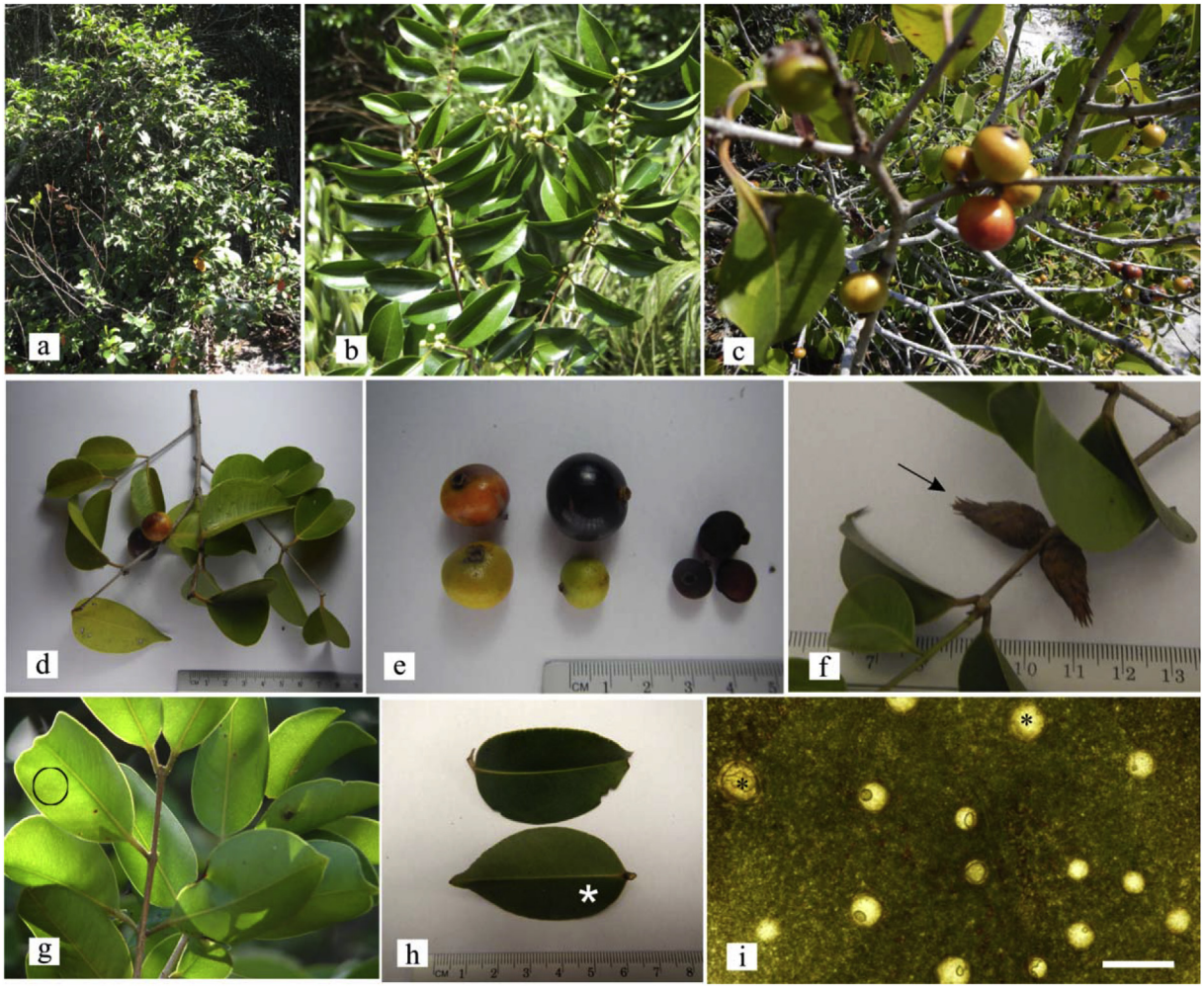


**Fig. 1.** A. Arbustive habit of a specimen of *Neomitranthes obscura* collected in the Marambaia *restinga* (RJ). B-H. Aspects of *N. obscura* morphology. B. Flowery branches (March 2015). C-E. Detail of the reproductive axis showing different developmental stages of green, yellow and black fruit, as well as dry fruit, collected in the Massambaba *restinga* (Araruama) in March 2015 (E). F. Specific gall formation (Cecidomyiidae, *Neomitranthella robusta*) in specimens collected in Massambaba. G. Leaves against the sun allow visualization of the points of secretory cavities (circle). H. Ovate or elliptic leaves, apex acuminate, cuneate base (Souza and Morim, 2008), *abaxial face. I – Secretory structures* in leaves contain essential oils. Bar = 200 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

*N. obscura* presents large secretory cavities in leaves, a unique taxonomic feature responsible for the accumulation of important plant volatiles involved in plant defense (Fig. 1g and i) (Souza and Morim, 2008; Victório et al., 2011; Arruda and Victório, 2011). Considering tropical ecosystems, some studies also suggest that a high proportion of species accumulate terpenes and that this could possibly be associated with protection against temperature or radiation, or with signaling and communication among species in the ecosystem (Sardans et al., 2015).

*Restingas* are sandy coastal plains forming large areas of sediment dating from the Quaternary period. In such areas, microclimatic variations can affect the composition of plant species and their chemical profile (Suguio and Tessler, 1984). In Brazil, about 79% of coastline is influenced by winds and salinity of the Atlantic Ocean, which, together with soil fertility, can affect plant life inhabiting *restingas*. This tropical ecosystem consists of a mosaic of vegetation types that exhibit structural differences, ranging from flora with herbaceous features to shrub formations. Many *restingas* in Rio de Janeiro are officially protected areas.

This work aimed to evaluate the composition of leaf essential oils in *N. obscura* from different *restingas* of Rio de Janeiro State under such variable environmental conditions as temperature, precipitation and topography. Studies of essential oil composition of plants collected in different places and seasons are important to detect the sources of highest metabolite content. We also evaluated the secretory cavities and their contents via microscopic analyses of the leaves. *N. obscura* is an endemic plant at risk of extinction as a consequence of increasing anthropogenic activities in *restinga* areas. Accordingly, this work is part of a project to study and protect the natural resources within *restingas*, as well as Myrtaceae species, in Rio de Janeiro State.

## 2. Material and methods

### 2.1. Plant material and study area

Samples of *N. obscura* were collected in the *restingas* of Marambaia (Rio de Janeiro City), Maricá (Maricá City), Jaconé (Maricá/Saquarema City) and in the Massambaba Environmental Protection Area (Araruama City), all in Rio de Janeiro State (Fig. 2). Table 1 shows the locations (latitude and longitude), average annual precipitation and temperature of the collection areas. Leaf samples were collected in June