TORI IN SPECIES OF DIARTHRON, STELLERA AND THYMELAEA (THYMELAEACEAE)

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SUMMARY

Torus thickenings have been found previously in intervacular pit membranes of species of Daphne and Wikstroemia (Thymelaeaceae). A search for tori was undertaken in the closely related genera Diarthron, Stellera and Thymelaea. Tori were observed in five of the seven species of Diarthron that were investigated. Presence of tori was associated with commonly occurring imperforate conducting elements and with perennial growth habit. Tori of a different morphology from that of Diarthron were present in two of the three specimens of Stellera chamaejasme that were studied. This study suggests torus presence to have systematic value; specifically, tori are present in species of the subgenera Dendrostella and Stelleropsis within Diarthron but absent in the subgenus Diarthron. Of 19 species of Thymelaea investigated, only two of four specimens of T. villosa contained torus-bearing pit membranes. It is suggested that the original classification of this species as Daphne villosa be reconsidered.

Key words: Diarthron, Stellera, Thymelaea, pit membrane, torus.

INTRODUCTION

A torus is a centrally located thickening in intervacular pit membranes of wood. Once thought to be rare in angiosperm woods, tori are now known from close to 80 species of eudicots (Dute et al. 2010). Ohtani and Ishida (1978) first discovered tori in three species of Daphne within the Thymelaeaceae. Subsequent studies found tori in many (but not all) species of Daphne and in the sister genus Wikstroemia (Ohtani 1983; Dute et al. 1992, 1996). No tori were found in species from some other genera of the Thymelaeaceae, such as: Drapetes, Edgeworthia (Dute et al. 1996), Gnidia, Dirca and Pimelea (Dute et al. 2001).

In 2002 Van der Bank et al. provided a molecular phylogeny of the Thymelaeaceae using rbcL nucleotide sequences. The results of this investigation showed Daphne and Wikstroemia to be closely related to the genera Thymelaea, Stellera and Diarthron. If other torus-bearing woods occur in the Thymelaeaceae, the latter three genera would be a good place to initiate the search. A similar situation exists in the Oleaceae where species of Osmanthus were known to have tori (Ohtani & Ishida 1978; Dute & Rushing 1987; Rabaey et al. 2006). A molecular study (Wallander & Albert 2000) identified...
the closest relatives of *Osmanthus* and was instrumental in a successful search for tori in the related genus *Picconia* (Dute et al. 2008; Rabaey et al. 2008).

The present manuscript details the results of a survey for tori done in species of *Thymelaea, Stellera* and *Diarthron* (sensu Tan 1980, 1982).

**MATERIALS AND METHODS**

Herbarium specimens used in this study are listed in Table 1. Slivers of tissue were taken with a razor blade either along the stem axis or at the site where stem meets root.

Table 1. Specimens of Thymelaeaceae sampled in this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Herbarium acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. lessertii (Wikstr.)</td>
<td>K, K, K, K</td>
</tr>
<tr>
<td>D. stachyoides</td>
<td>L*</td>
</tr>
<tr>
<td>Diarthron lessertii (Wikstr.)</td>
<td>K, K, K, K</td>
</tr>
<tr>
<td>D. linifolium Turcz.</td>
<td>L*</td>
</tr>
<tr>
<td>D. magakjanii (Sosn.) Kit Tan</td>
<td>E, E</td>
</tr>
<tr>
<td>D. vesiculosum (Fisch. &amp; C.A. Mey. ex Kar. &amp; Kir.)</td>
<td>L*, E, L, E</td>
</tr>
<tr>
<td>Stellera chamaejasme L.</td>
<td>L*, E, E</td>
</tr>
<tr>
<td>S. circinata Lecomte</td>
<td>L*</td>
</tr>
<tr>
<td>S. fargesii Lecomte</td>
<td>L*</td>
</tr>
<tr>
<td>S. lessertii (Wikstr.)</td>
<td>K, K, K, K</td>
</tr>
<tr>
<td>S. stachyoides Schrenk</td>
<td>K</td>
</tr>
<tr>
<td>Stelleropsis magakjanii (Sosn.)</td>
<td>Pobed.: E, anon. s.n. —</td>
</tr>
</tbody>
</table>
Table 2. Generic classification and growth habit of *Diarthron* and *Stellera* as recognized by Tan (1982) except as noted.

Sampled specimens are marked by the presence or absence of tori (Y = present; Y-N = torus present in some specimens; N = absent) and by tracheids (imperforate conducting elements with numerous bordered pits; C = common; U = uncommon; A = absent).

**Diarthron** Turcz.

**Subg. Dendrostella** (C.A. Meyer) Kit Tan – suffrutescent or shrubs

- *Diarthron arenaria* (Pobed.) Kit Tan - Y/C (syn. *Dendrostella arenaria* Pobed.)
- *Diarthron lessertii* (Wikstr.) Kit Tan - Y/C (syns. *Chamaejasme lessertii* (Wikstr.) Kuntze; *Dendrostella lessertii* (Wikstr.) C.A. Mey.)
- *Diarthron linearifolia* (Pobed.) Kit Tan (syn. *Dendrostella linearifolia* Pobed.)
- *Diarthron macrorhachis* (Pobed.) Kit Tan (syn. *Dendrostella macrorhachis* Pobed.)
- *Diarthron olgae* (Pobed.) Kit Tan (syn. *Dendrostella olgae* Pobed.)
- *Diarthron ramosissima* (Pobed.) Kit Tan (syn. *Dendrostella ramosissima* Pobed.)
- *Diarthron stachyoides* (Schrenk) Kit Tan - Y/C (syns. *Chamaejasme stachyoides* (Schrenk) Kuntze; *Dendrostella stachyoides* (Schrenk) Tiegh.; *Stellera stachyoides* Schrenk)
- *Diarthron turkmenorum* (Pobed.) Kit Tan (syn. *Dendrostella turkmenorum* Pobed.)

**Subg. Diarthron** - annual herbs

**Sect. Arthrochlamys** C.A. Meyer

- *Diarthron vesiculosum* (Fisch. & C.A. Mey. ex Kar. & Kir.) C.A. Meyer - N/U (syns. *Diarthron carinatum* Joub. & Spach; *Passerina costata* Griff.; *Passerina vesiculosa* Fisch. & C.A. Mey. ex Kar. & Kir.)

**Sect. Diarthron**


**Subg. Stelleropsis** (Pobed.) Kit Tan - suffrutescent

**Sect. Stelleropsis**

- *Diarthron isykkulensis* (Pobed.) Kit Tan (syn. *Stelleropsis isykkulensis* Pobed.)
- *Diarthron tarbagataica* (Pobed.) Kit Tan (syn. *Stelleropsis tarbagataica* Pobed.)
- *Diarthron tianschanica* (Pobed.) Kit Tan (syns. *Stelleropsis altaica* Pers. subsp. *tianschanica* (Pobed.) Kamelin; *Stelleropsis tianschanica* Pobed.)

**Sect. Turcomanica** (Pobed.) Kit Tan

- *Diarthron antoninae* (Pobed.) Kit Tan (syn. *Stelleropsis antoninae* Pobed.)
- *Diarthron caucasica* (Pobed.) Kit Tan (syn. *Stelleropsis caucasica* Pobed.)
- *Diarthron iranica* (Pobed.) Kit Tan subsp. *iranica* (syn. *Stellera altaica* Pers. var. minor Boiss.)
- *Diarthron magakjanii* (Sosn.) Kit Tan - Y/C (syns. *Stellera magakjanii* Sosn.; *Stelleropsis magakjanii* (Sosn.) Pobed.)
- *Diarthron turcomanica* (Czerniak.) Kit Tan - Y/C (syns. *Stellera turcomanica* Czerniak.; *Stelleropsis turcomanica* (Czerniak.) Pobed.; *Wikstroemia turcomanica* (Czerniak.) Domke)

**Stellera** L. - suffrutescent

- *Stellera chamaejasme* L. – Y-N/C (syns. *Chamaejasme stelleriana* Kuntze; *Wikstroemia chamaejasme* (L.) Domke)

**Taxa not treated by Tan (1982)**

- *Stellera circinata* Lecomte - N/C (syns. *Diplomorpha dolichantha* (Diels) Hamaya; *Wikstroemia circinata* (Lecomte) Domke; *Wikstroemia dolichantha* Diels; *Wikstroemia effusa* Rehder)
- *Stellera fargesii* Lecomte - N/A (syns. *Daphne fargesii* (Lecomte) Halda; *Wikstroemia fargesii* (Lecomte) Domke)
Slivers were placed in 95% ethanol under vacuum for 30 minutes. The material then was placed in fresh 95% ethanol and left to soak overnight. Alcohol was replaced with three changes of JB-4 resin over a 24-hour period before polymerization occurred. Transverse sections of 3 to 10 μm in thickness were cut on either a JB-4 microtome or on a Sorvall MT-2b ultramicrotome. Those specimens of Diarthron and Thymelaea found to possess tori were later cut to form tangential longitudinal sections. Sections were heat-fixed to slides, stained with benzoate-buffered, aqueous toluidine blue O, embedded in Permount mounting medium (Fisher Scientific, Fair Lawn, New Jersey, U.S.A.) and topped with a coverslip. Photographs were taken with a Nikon D-70 digital camera attached to a Nikon Biophot microscope or with a Nikon Eclipse 80i epifluorescence microscope (used under brightfield settings) with a Qimaging Fast 1394 digital camera.

For the purpose of identifying tracheids vs vessel members, macerated material was prepared by the method of Wheeler (1983) using equal parts of glacial acetic acid and hydrogen peroxide at 50°C for three days. Subsequently, specimens were washed repeatedly in de-ionized water and stained with 1% buffered toluidine blue O (TBO) before observation.

RESULTS

Diarthron and Stellera

Tan in 1982 provided a classification of Stellera and Diarthron based on morphology. Her results are presented in Table 2, and her classification is used in the present manuscript. Stellera circinata and S. fargesii are appended to the bottom of the table as they were not originally included in Tan’s list of species. In fact, these two species have been placed in Wikstroemia and other genera (Domke 1932; Multilingual Multiscript Plant Name Database 2005; Rogers 2009; Tropicos 2010). Presence or absence of tori is indicated on the table. Although the number of species investigated is by no means comprehensive, preliminary evidence shows torus presence to be correlated with taxonomy; specifically, tori are present in species of the subgenera Dendrostella and Stelleropsis within Diarthron but absent in the subgenus Diarthron. Tori are also present in the genus Stellera and are discussed later in the manuscript.

Tori of Diarthron are seen as lens-shaped structures in sectional view and as circular in face view (Fig. 1–3). They are associated with circular pit borders with circular to oval pit apertures (Fig. 3). Sometimes the torus is distinguishable as two separate pads separated by a faint middle lamella, but such images are difficult to capture using light microscopy. Generally, tori are aspirated (as would be expected in air-dried specimens) but can also be found in a non-aspirated condition (Fig. 1). In cross-sectioned material, tori are common in bordered pit pairs of latewood between two narrow diameter tracheary elements (Fig. 1 & 2) and between vessel members and such tracheary elements (Fig. 2). Sometimes, as in Diarthron turcomanica, one can distinguish in cross-sectioned material even small diameter vessel members from tracheids. This distinction is determined by wall thickness, lumen diameter and staining properties but is not always apparent in other species unless a small perforation is observed. As a result, the small diameter conducting elements in cross-sectioned material are referred to simply as
narrow diameter tracheary elements to distinguish them from larger diameter conducting elements that can be unambiguously distinguished as vessel members. Some species do not have tori in their earlywood, but others, such as *Diarthron turcomanica*, *D. arenaria* and *D. magakjanii* do. In these species earlywood tori are found between narrow diameter tracheary elements and in the latter two species between narrow diameter tracheary elements and vessel members. In no species possessing tori were these structures found in pit membranes between two large diameter vessel members (Fig. 4).

Figures 1–4. Intervascular pit membranes in *Diarthron* spp. – 1: Latewood narrow diameter tracheary elements with tori, both aspirated (arrow) and nonaspirated (double arrow), *D. arenaria*, XS. – 2: Latewood of *D. arenaria* showing tori connecting narrow diameter tracheary elements (arrow) as well as narrow diameter tracheary elements to vessel members (double arrow). The vessel members are distinguished by their large diameters and perforations, XS. – 3: *D. lessertii* wood showing circular bordered pits containing tori (arrows). Oval apertures are also visible (double arrows), TLS. – 4: Earlywood vessel members of *D. magakjanii*. Tori are absent from the bordered pit pairs (arrows). — Scale bars = 10 \( \mu m \).
Tangential longitudinal sections allow one to distinguish between tracheids and vessel members. In such sections, tori exist between adjacent tracheids and between tracheids and vessel members in the latewood. Tori are seldom found between tracheids and large diameter earlywood vessel members and are not found (with one exception) between vessel members.

Macerations of *Diarthron* woods show that a correlation exists between tori and the common occurrence of tracheids (Table 2). The subgenera *Dendrostellera* and *Stelleropsis* contain species whose woods possess both tori and readily identifiable tracheids. For example, the ratios of vessel members to tracheids in macerations of *Diarthron lessertii* (Leiden) and *Diarthron magakjanii* (Edinburgh) are 2.1 to 1 (N = 286) and 0.5 to 1 (N = 723), respectively. Woods of the subgenus *Diarthron* have no tori, and a maceration of *D. linifolium* wood shows a ratio of 22 to 1 (N = 574). In species of *Dendrostellera* and *Stelleropsis*, tracheids are either associated with vessel members (Fig. 5) or are in linear arrays probably representing radial files of latewood elements (Fig. 6).

Figures 5 & 6. Macerations of *Diarthron turcomanica* wood. – 5: Vessel member (left) and two tracheids. – 6: A series of tracheids. — Scale bars = 25 μm.

Possible torus-bearing pit membranes are present in the two Edinburgh specimens of *Stellera chamaejasme* but not in the one received from Leiden. The typical morphology of such pit membranes is one in which a middle lamella is sandwiched between two distinct clear areas. Each clear area is in turn covered by a deeply staining frequently curved pad of material (Fig. 7 & 8). Sometimes only one pad is visible or the pads are of two different diameters. The torus morphology in *Stellera chamaejasme* resembles somewhat the structure of pseudotori in pit membranes of the Rosaceae (Parameswaran
& Liese 1981; Jansen et al. 2007), but we could observe no attachment of the thickenings to the periphery of the pit membrane. A TEM study is needed to resolve the precise nature of these thickenings.

Figures 7 & 8. Intervascular pit membranes in *Stellera chamaejasme*, XS. Note the tori (arrows), each with two distinct pads. — Scale bars = 10 μm.

None of the species of *Thymelaea*, except *T. villosa*, have tori (Table 3). Even for this species tori were located only in specimens collected by Galicia-Herbada and Reverchon (Fig. 9 & 10). Specimens collected by Hegnauer and Pitard lacked them (q.v. Table 1 & Table 3). The specimens collected by Van den Bosch had been heavily damaged by fungi and no determination could be made.

Tori are found only rarely in earlywood. The latewood tracheary elements consist of tracheids and narrow-diameter vessel members as noted in tangential longitudinal sections. It is in the latewood that the tori are generally observed. Even in the latewood of

*Thymelaea*

Figures 9 & 10. Latewood of *Thymelaea villosa* (XS) showing tori (arrows). — Scale bars = 5 μm.
torus-bearing specimens the torus is not found in every bordered pit pair. For example, in the Galicia-Herbada specimen only 26% (N = 100) of the latewood bordered pit pair membranes possessed tori.

A trans-section through the base of the stem of a specimen of *T. passerina* collected in Austria (AUA–Barta 2007-75) showed two growth rings in a species thought to be an annual (Fig. 11).

**DISCUSSION**

**Diarthron and Stellera**

The debate regarding tracheid terminology is contentious (Carlquist 1984, 1985). Certainly, the tracheids of *Diarthron* and *Stellera* do not fit the definition of Carlquist’s “true tracheids”
as fibers also exist in the wood. Mcalpine and Chalk (1950) considered wood of three genera of Thymelaeaceae (Daphne, Dirca and Ovidia) to possess both vasicentric and vascular tracheids. Carlquist (1985), in contrast, generally considered this family to have vasicentric tracheids. We are hesitant to define precisely the tracheid type without a separate study to that end.

Classification of the genera Stellera and Diarthron is confusing. This confusion can be observed in Table 1 where, for example, one species has been sent to us under three different binomials (Dendrostella lessertii, Diarthron lessertii, Stellera lessertii). Tan (1982) took the previously separate genera Diarthron (annual herbs), Dendrostella (shrubs and suffrutices) and Stelleropsis (suffrutices) and reduced them to subgenera within the genus Diarthron. Stellera, a monotypic genus, was considered to be the sister genus of Diarthron. Stellera and Diarthron sensu lato were distinguished by floral and inflorescence characters. Wang and Gilbert (2007a, b) accepted Tan’s basic classification in the Flora of China although they noted that there were 10 to 12 species of Stellera, but only S. chamaejasme existed in China. Using Tan’s classification as a guide, it appears as if the presence of tori in Diarthron is correlated not only with the frequent presence of tracheids, but also with growth habit. Tori were absent from the annuals D. linifolium and D. vesiculosum, and as expected, they did not have growth rings (the samples were collected from slightly above the root collar). Diarthron stachyoides, although a perennial, also showed no multiple rings, but it was sampled from a small diameter aerial branch. These results regarding torus distribution are tentative in that more species must be sampled and each specimen should be sampled along the length of the root-stem axis.

Both presence and absence of tori from different species within a genus of the Thymelaeaceae is not unique to Diarthron. In Daphne all sections save one (Mezereum) have tori (Dute et al. 1992, 1996). In the genus Wikstroemia, tori were observed in three of seven species investigated from the subgenus Diplomorpha, from one species of the subgenus Daphnimorpha, and absent from the three species in the subgenus Wikstroemia (Dute et al. 1996).

Recent molecular studies have called into question Tan’s classification. Van der Bank et al. (2002), using plastid DNA analysis, noted that Diarthron and Stellera were not nearest neighbors. Rather, Stellera shared a clade with Wikstroemia, and Diarthron shared one with Daphne/Thymelaea. Further molecular investigations using the ITS region of nuclear DNA (Galicia-Herbada 2006) showed Diarthron to be polyphyletic and that Dendrostella and Stelleropsis should regain generic status. Such a change would restrict the non-torus-bearing species to the diminished genus Diarthron.

**Thymelaea**

An explanation for the situation with T. villosa is elusive. The basionym for the species is Daphne villosa L. According to Tan (1980), Thymelaea is more closely related to Daphne than to other neighboring genera based on floral morphology. This relationship was confirmed by Van der Bank et al. (2002) and by Galicia-Herbada (2006) using molecular techniques. Tori were present in all sections of Daphne save one (Dute et al. 1992, 1996), and perhaps T. villosa should have remained in that genus. This solution...
does not explain why tori were found in two of the four species, however. A second possibility is that there are two nearly identical species, one with and one without tori. All specimens came from stems or stem bases so that the argument cannot be made that the torus is located in one organ and not in another.

*Thymelaea passerina* is of interest as an invasive in the United States. Its native range includes the Mediterranean region of Europe as well as East and Central Asia (Tan 1980; Galicia-Herbada 2006). It is considered an annual found in dry environments such as “eroded slopes, dry river beds and salt-flats” (Tan 1980). According to Tan, seeds probably were introduced into Australia and North America among fodder. The species has spread rapidly in the United States no doubt due to its system of inbreeding where even individual plants can set seed (Tan 1980). The first account of the plant in the U.S. was published by Pohl in 1955 from specimens located in Nebraska and Iowa. Since that time populations have been discovered throughout the Midwest (Vincent & Thieret 1987; Kostel 2009) as well as the Southeast (Wofford & DeSelm 1988; Webb et al. 1997) and the state of Washington (Washington State NWCB 2008). In contrast to published reports that *T. passerina* is an annual, a cross section through the base of the stem of a specimen collected in Austria (AUA–Barta 2007-75) showed two growth rings (Fig. 11). Provided the plant identification is correct, the notion that this species is strictly an annual is called into question.

**Ecology and Evolution**

It has been hypothesized that the presence of tori in intervascular pit membranes of *Daphne* and *Wikstroemia* might prevent rupture of the pit membrane during aspiration (Dute et al. 1990, 1996). It was felt that narrow diameter, torus-bearing tracheary elements served as a back-up system to relatively large-diameter vessel members (Dute et al. 1996). Such a system would be of distinct advantage in dry habitats. The Thymelaeaceae as a whole are associated with such environments (Rendle 1925). Species of *Diarthron* and *Stellera* grow in the steppe regions of central and eastern Asia and are exposed to both winter cold and summer drought (Tan 1982).

Species of *Thymelaea* occur primarily in the Mediterranean region, localized mainly in northwest Africa and southwest Europe (Tan 1980). The one exception to this generalization is, as previously mentioned, *T. passerina*, which extends well into Central Asia (Tan 1980). *Thymelaea* is associated with xeric environments. Of special note is *T. hirsuta*, a potential fiber crop (Shaltout 1992), which grows in sand dunes in the western Mediterranean region of Egypt (Shaltout 1992) as well as in the desert of Beersheba, Israel (Fahn & Sarnat 1963). Many of the species of *Thymelaea* are associated with well-drained sandy or rocky soils (Tan 1980). Galicia-Herbada (2006) hypothesized that *Thymelaea* and *Daphne* diverged in the Lower or Middle Miocene during a time of increasing aridity with *Thymelaea* being the more xeromorphic of the two genera. However, with the exception of *T. villosa*, it is the genus *Daphne* that possesses tori. If the common ancestor of both genera had tori, why is it missing from most species of *Thymelaea*? If, due to a fortuitous mutation (or set of mutations), tori arose in *Daphne* after the split from *Thymelaea*, then the case for *T. villosa* being returned to *Daphne* is strengthened.
Structure and Function

Study of the structural/functional aspects of intervascular pit membranes of the Thymelaeaceae are few and are restricted largely to Daphne. Mean torus diameter in a number of Daphne species is greater than the average maximum diameter of the associated aperture (Dute et al. 1990, 1992, 2001). For example, in D. gnidioides Jaub. & Spach torus diameter is 2.77 μm (2.28–3.29, N = 25), whereas the long axis of the aperture is 1.62 μm (1.05–2.22, N = 25) (Dute et al. 2001). Therefore, provided pit deflection is complete during aspiration, occlusion of the aperture by the torus should be complete. In addition, the circularity ratio (i.e. ratio of short axis to long axis of the aperture) was 0.71 for a specimen of D. gnidioides (N = 25). The circularity ratio for two specimens of Gnidia caffra (Meisn.) Gilg were 0.26 and 0.28, for two specimens of Pimelea arenaria A. Cunn. 0.29 and 0.36, for P. prostrata Willd. 0.3. Neither Gnidia nor Pimelea has tori. However, Dirca palustris L., also a species without tori, had ratios of 0.73, 0.78 and 0.63 for three specimens.

Hacke et al. (2004) found that pit conductivity in pit membranes of gymnosperms was maximized by “tight scaling” between torus and pit size. Specifically, the torus diameter, membrane diameter and aperture diameter all are important in maximizing pit conductivity per pit membrane area. These factors are used to determine the “torus overlap,” which is the diameter of the torus minus the diameter of the aperture divided by the diameter of the membrane minus that of the aperture (Hacke et al. 2004). A torus overlap of between 0.21 and 0.38 was calculated for gymnosperms. Interestingly, calculations using torus-bearing pit membranes of Cercocarpus, an angiosperm, found a mean value for vessel members of 0.26 and for tracheids of 0.30 (Dute et al. 2010). Also, the torus in Cercocarpus was 46% of the pit diameter, compared to 48% for three conifer species (Hacke & Jansen 2009) and 45% for earlywood tracheids of Pseudotsuga menziesii (Domec et al. 2006). It would be interesting to do similar calculations with species of Diarthron and Daphne (say Diathron lessertii and Daphne gnidioides) to see whether the previous generalizations hold true. To make such calculations would require accurate measurements using either scanning electron microscopy (SEM) and/or atomic force microscopy (AFM). The latter has already been used to visualize torus-bearing pit membranes in the wood of Osmanthus armatus Diel (Jansen et al. 2010).

According to Jansen et al. (2009), thicker pit membranes are less porous than thinner ones which in turn leads to higher air-seeding thresholds in the former. It would be useful to compare thicknesses of margo vs. torus in Daphne and Diarthron species. Ultra-thin sections of pit membranes viewed with TEM would suffice for this task. However, specimens would have to be preserved in the hydrated condition since air-drying the pit membranes prior to processing leads to a decrease in thickness (shrinkage) (Dute 1994).

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