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COMPARATIVE ANATOMY OF STEM AND LEAF OF *RICOTIA* L. GROWING IN TURKEY

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Abstract

The present paper deals with a comparative anatomy of stem and leaf of *Ricotia* species growing in Turkey. In addition, stomatal index and rate of these species were calculated. A diagnostic key based on combined stem, leaf and leaf epidermal characteristics were presented. The presence or absence of trichome, cortex parenchyma layer, sclerenchyma, mesophyll structure and epidermal surface were found to be important characters for the identification of *Ricotia* species.

Introduction

Brassicaceae is one of the largest families of angiosperm comprising 338 genera and 3709 species and distributed throughout the world, mainly in temperate regions of the Northern Hemisphere (Al-Shehbaz 1984, Warwick *et al.* 2006, Kasem *et al.* 2011). The major centres of distribution of the family are in the Irano-Turanian, Mediterranean and Saharo-Sindian regions (Hedge 1976). Turkey is one of the richest countries in the world in terms of the number of species of the Brassicaceae represented by 571 species 65 subspecies, 24 varieties and approximately 660 taxa belonging to 91 genera (Al-Shehbaz *et al.* 2007). The genus *Ricotia* is represented by nine species which are distributed in the South East Europe, Eastern Mediterranean and adjacent Middle East (Burtt 1951, Appel and Shehbaz 2003). In the flora of Turkey, there are 6 species (*Ricotia tenuifolia* Sibth. & Sm., *R. sinuata* Boiss. & Heldr., *R. carnosula* Boiss. & Heldr., *R. davisiana* B.L. Burtt., *R. varians* B.L. Burtt. and *R. aucheri* (Boiss.) B.L. Burtt). *Ricotia* has five endemic species in Turkey. The rate of endemism of *Ricotia* species in the flora of Turkey is 83.3% (Davis 1985).

Metcalfe and Chalk (1957) studied the anatomy of the family Brassicaceae and determined the diagnostic anatomical characteristics as epidermal cell type, stoma type and the arrangement of the sclerenchymatic cells around the vascular bundles of the leaves.

The anatomical properties of *Ricotia* have not so far been studied. Therefore, the purpose of this study was to investigate the anatomical properties as well as to find out anatomical peculiarities of different species of *Ricotia*.

Materials and Methods

Plant specimens were collected from different localities in Turkey and had been submitted in Tunceli University Herbarium (Table 1). Anatomical studies were carried out on specimens preserved in 70% alcohol. Cross-sections of stem and leaves were stained with phloroglucinol-HCl solutions (Yakar-Tan 1982) and chlorophyll in leaves was removed with chloral hydrate.

Stomatal density on abaxial and adaxial surfaces of the leaf was counted under a light microscope. Stomatal index was calculated according to the method of Meidner and Mansfield (1968). Stomatal terminology and the leaf epidermal terminology were based on the classification

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proposed by Dilcher (1974) and Wilkinson (1979), respectively. Measurements and photographs were taken using Olympus BX 51 and Nikon Eclipse E600 binocular light microscopes.

Table 1. Specimens used for anatomical studies and localities of specimen collection.

Species	Habitat, altitute, date of collection and collector's number
R. tenuifolia	Antalya; between Finike and Elmalı, Calcareus rocky, 390 m, 24.04.2010, Paksoy 1080
R. *sinuata*	Antalya; Kemer, Tahtalı mount, Cableway station, roadsides, 100 m, 24.04.2010, Paksoy 1078
R. carnosula	Antalya; Kemer, Göynük canyon, around Göynük creek, calcareus slopes, 10 m, 24.04.2010, Paksoy 1075
R. davisiana	Antalya; Kemer, Tahtalı mount, Peynirlik location, 1600 m, 17.07.2010, Paksoy 1098
R. varians	Isparta; Aksu, Dedegöl mount, Obruk plateau, 1350 m, 01.08.2010, Paksoy 1104
R. aucheri	Kahramanmaraş; Çağlayancerit, Öksüz mount, Akdut location, calcareous mobile slopes, 1200 m, 11.06.2011, Paksoy 1094

Results and Discussion

Comparative anatomical characters of stem and leaf of *Ricotia* species are given in Tables 2 and 3, respectively. Table 4 shows the features of the leaf epidermis of *Ricotia* species.

Table 2. Stem anatomical characters of *Ricotia.*

Taxa	Presence/absence of trichomes	Cortex		Pericycle	İnterfascicular region	Phloem
		Parenchyma	Endodermis			
R. tenuifolia	Absent	3 - 5	1	1 - 2	Unclearly	2 - 5
R. sinuata	Present (sparsely)	3 - 4	1	1 - 2	Unclearly	3 - 5
R. carnosula	Absent	3 - 4	1	1	Unclearly	2 - 5
R. davisiana	Present (densely)	8 - 10	1	1	Clearly	2 - 6
R. varians	Absent	6 - 10	1	1 - 2	Clearly	2 - 6
R. aucheri	Absent	6 - 10	1	2 - 4	Clearly	3 - 11

Table 3. Leaf anatomical characters of *Ricotia.*

Taxa	Presence/absence of trichomes	Mesophyll type	Palisade layer	Spongy layer	Middle vascular bundle	
					Sclerenchyma	Bundle sheath
R. tenuifolia	Absent	Bifacial	1 - 2	3 - 5	Absent	Present
R. sinuata	Present (sparsely)	Bifacial	2 - 3	2 - 4	Absent	Present
R. carnosula	Absent	Bifacial	1 - 2	3 - 5	Absent	Present
R. davisiana	Present (densely)	Equifacial	1 - 2	2 - 4	Absent	Present
R. varians	Absent	Equifacial	2 - 4	1 - 2	Absent	Present
R. aucheri	Absent	Equifacial	2 - 4	2 - 3	Present	Present

Stem anatomy: In a cross section, the single-layered epidermis was found with a very thin cuticle on the outside (0.5 - 1.5 µm). The surface was covered with simple hairs (1 - 4 cells) or without hairs. Amphistomatic or epistomatic type of stomata were present on the epidermis. Cortex layer consists of parenchyma 3 - 10 layers, thin-walled, with regularly oval or round shaped cells. Cortex parenchyma also contained starch granules. Underneath cortex parenchyma, there was a single layered rectangular or oval shaped endodermis. Pericycle is underneath

endodermis. It was usually sclerenchymatic and surrounding on vascular bundles or cup-like 1-3 layers on phloem. The number of vascular bundles arranged in a ring is 7 - 16. Interfascicular region was located in between vascular bundles. Phloem was 4 - 7 layers and consisted of irregular and squashed cells. Cambium cells were not clear. Pith region occurred in the center of the stem composed of large orbicular or polygonal parenchymatic cells.

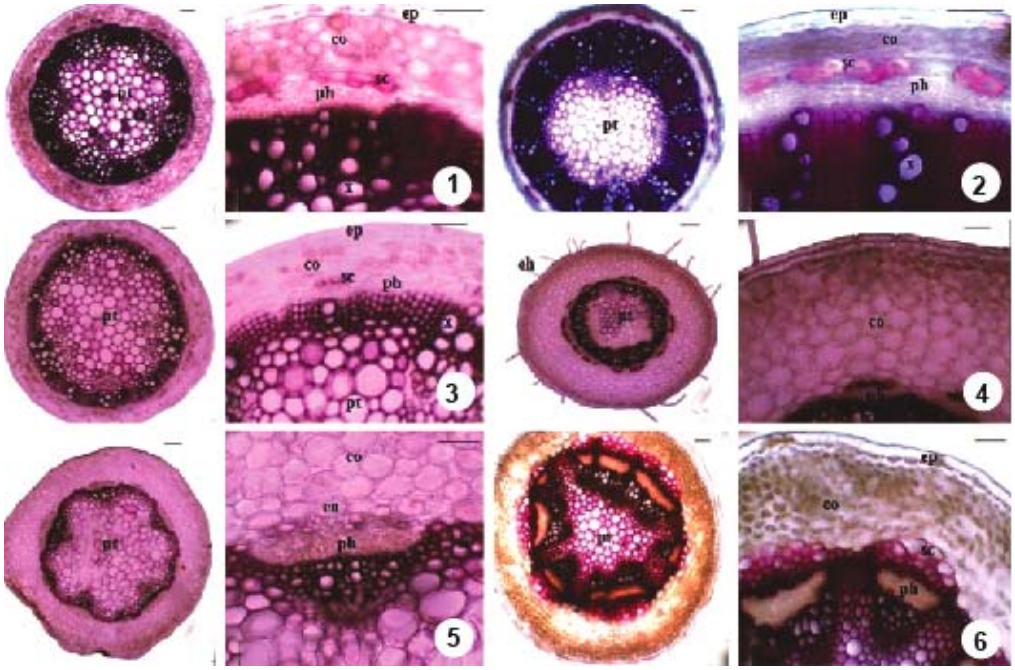

Fig. 1. Cross-section of stem of *Ricotia* species. 1. *R. tenuifolia*, 2. *R. sinuata*, 3. *R. carnosula*, 4. *R. davisiana*, 5. *R. varians*, 6. *R. aucheri*. cu: cuticle, ep: epidermal cell, sc: stomata cell. (Bars: 20 µm). co: cortex, eh: grandular hair, en: endodermis, ph: phloem, pt: pith and x: xylem.

Leaf anatomy: In the cross section of the leaf, there was a thin cuticle on the upper and lower epidermis. Both epidermal cells was isodiametric and rectangular, oval or cubic in shape. Surface of epidermis was covered with simple hairs (1 - 4 cells) or without hairs. Stomata were present on both surfaces of the leaf (amphistomatic type). These were raised above the surface or located on the same level as epidermal cells. In surface section, 3 different types of the epidermal cell walls were found which including large waves (sinuate), mild waves (undulate) or wave (straight). Stomata type was Cruciferous type (anisocytic) or rarely Ranunculaceous (anomocytic) type. The stomatal index is 21.1 - 33.3 (upper surface) and 15.8 - 31.9 (lower surface), while stomatal index ratio is between 0.6 and 1.43. Mesophyll consisted of palisade and spongy parenchyma cells. Palisade parenchyma cells were 1 - 4 layered, cylindrical whereas spongy parenchyma cells were 1 - 5 layered and round or oval in shape. Mesophyll were bifacial and equifacial. Vascular bundles were embedded in mesophyll and collateral type. They were surrounded by parenchymatic bundle sheath. Sclerenchymatic tissue on the bundles were present or absent. The xylem faces upper surface as phloem faces the lower epidermis. Sclerenchyma on phloem tissue was present or absent.

Table 4. Characteristics of the leaf epidermis of *Ricotia* species under light microscopy.

Characters	*R. tenuifolia*		*R. sinuata*		*R. carnosula*		*R. davisiana*		*R. varians*		*R. aucheri*	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Anticlinal cell wall	Undulate	Undulate	Sinuate	Sinuate	Straight	Undulate	Straight	Undulate	Straight	Straight	Straight	Straight
Shape of epidermal cells	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Polygonal	Irregular	Irregular	Irregular	Irregular	Irregular
Stomata length (µ)	24 - 26	22 - 29	17 - 23	18 - 24	20 - 31	23 - 33	20 - 28	16 - 38	17 - 24	17 - 26	32 - 46	36 - 40
Stomata width (µ)	16 - 21	19 - 22	17 - 19	16 - 21	15 - 21	14 - 20	20 - 27	16 - 24	14 - 20	15 - 21	28 - 39	26 - 37
Number of stomata (1 mm^2)	104	60	100	100	80	74	117	98	219	192	96	84
Number of epidermal cells	354	320	366	325	300	259	453	209	496	462	192	196
Stomata index	22.7	15.8	21.4	23.5	21.1	22.2	20.5	31.9	30.6	29.4	33.3	30
Stomata index ratio	1.43		0.9		0.95		0.6		1.04		1.11	

Ricotia species have been shown the typical features of the family Brassicaceae (Metcalfe and Chalk 1957). Anatomically, important characters for identification of *Ricotia* species were presence or absence of trichome, cortex parenchyma layer, presence or absence of sclerenchyma, mesophyll structure, and epidermal surface.

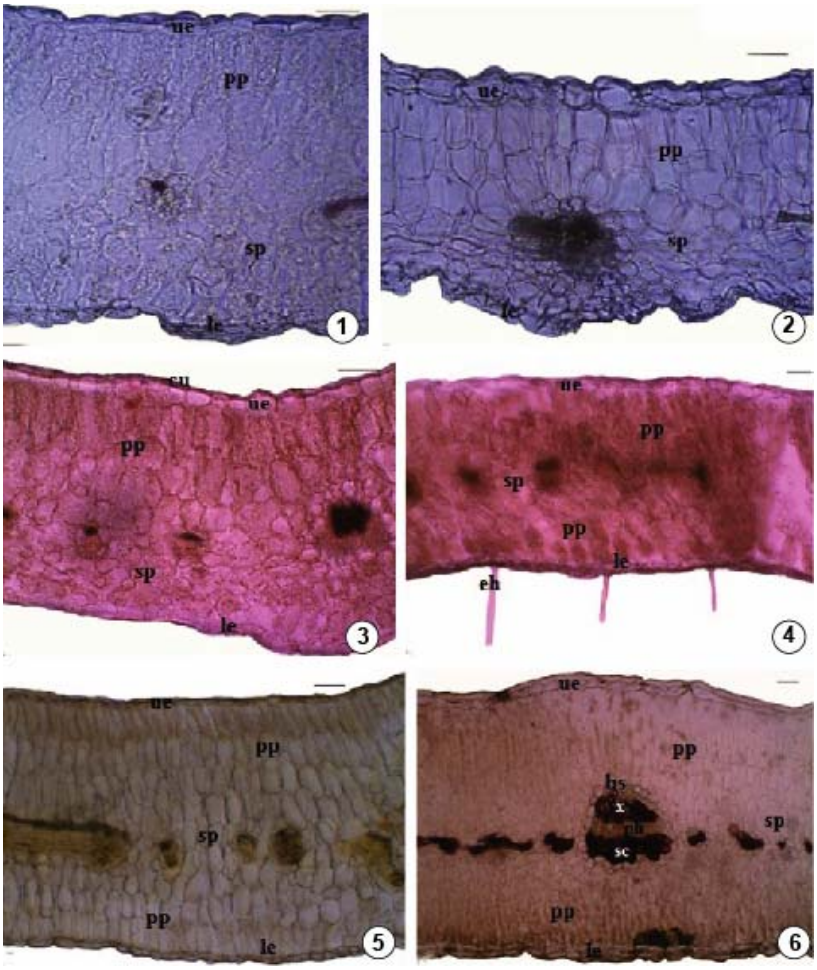

Fig. 2. Cross-section of leaves of *Ricotia* species. 1. *R. tenuifolia*, 2. *R. sinuata*, 3. *R. carnosula*, 4. *R. davisiana*, 5. *R. varians*, 6. *R. aucheri*. cu: cuticle, eh: eglandular hair, ue: upper epidermis (adaxial surface), pp: palisade parenchyma, sp: spongy parenchyma, bs: bundle sheath, x: xylem, ph: phloem, sc: sclerenchyma and le: lower epidermis (abaxial surface). (Bars: 20 µm).

The stomata of *Ricotia* species were surrounded by three subsidiary cells of which one was usually much smaller than the other two, the so-called Cruciferous (Anisocytic) type. Rarely stoma surrounded by a limited number of cells which were indistinguishable in size, shape, or form from those of the remainder of the epidermis, the so-called Ranunculaceous (Anomocytic) type (Fig. 5). Also, the stomatal index of these species varied from 21.1 - 33.3 (upper surface) and 15.8 - 31.9 (lower surface) and the stomatal index ration varied between 0.6 and 1.43. Three different types of the epidermal cell walls were seen. These are sinuate (*R. sinuata*), undulate

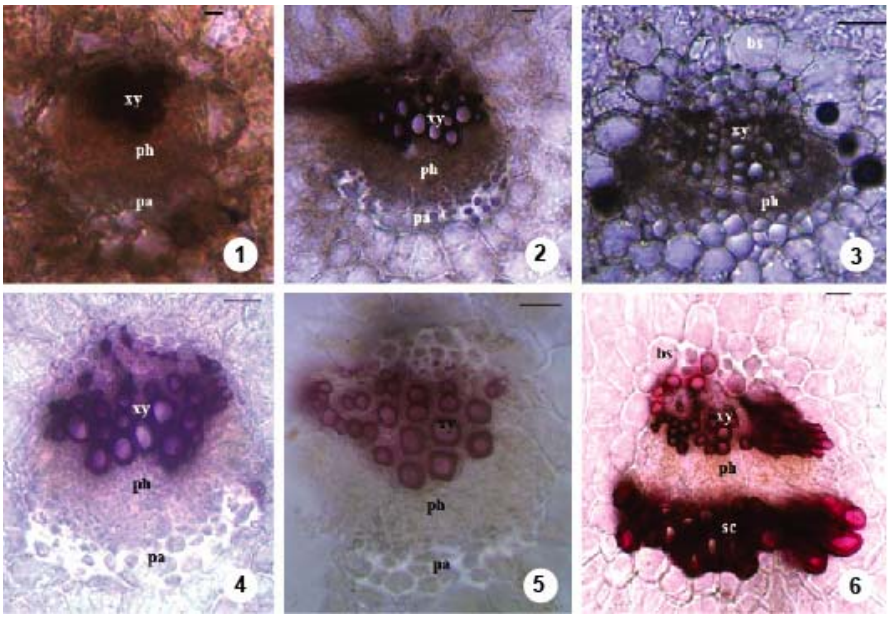

Fig. 3.Middle vascular bundles of leaves of *Ricotia* species. 1. *R. tenuifolia*, 2. *R. sinuata*, 3. *R. carnosula*, 4. *R. davisiana*, 5. *R. varians*, 6. *R. aucheri*. bs: bundle sheath, xy: xylem, ph: phloem, sc: sclerenchyma and pa: parenchyma (Bars: 20 µm).

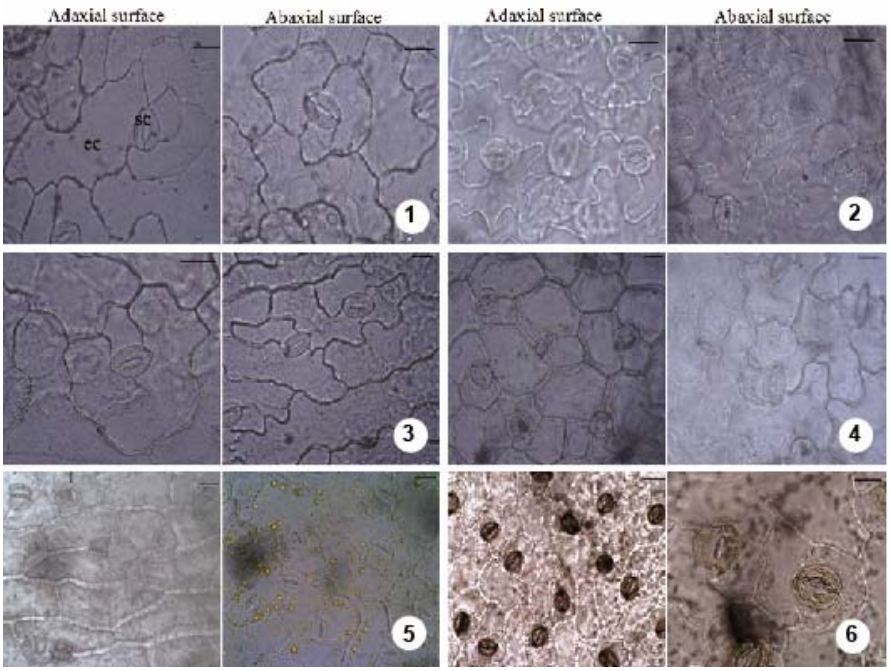

Fig. 4. Adaxial and abaxial epidermal surfaces of leaves of *Ricotia* species. 1. *R. tenuifolia*, 2. *R. sinuata*, 3. *R. carnosula*, 4. *R. davisiana*, 5. *R. varians*, 6. *R. aucheri*. cu: cuticle, ec: epidermis cell and sc: stomata cell. (Bars: 20 µM).

(*R. tenuifolia*, *R. carnosula* and *R. davisiana*) and straight (*R. carnosula*, *R. davisiana*, *R. varians* and *R. aucheri*) (Fig. 4). The pattern of mesophyll significant anatomical character for *Ricotia* species. *R. tenuifolia*, *R. sinuata* and *R. carnosula* are bifacial (dorsiventral) while *R. davisiana, R. varians* and *R. aucheri* are equifacial (isobilateral) (Fig. 2). *R. davisiana* can be easily separated anatomically from other species. One of the most important characters of this species is the trichome (simple, 1 - 4 celled) which are found densely on stem and leaf. Another important character is the presence of sclerenchyma on vascular bundle of leaf (Figs 1, 3). Anatomical characters of *R. tenuifolia* and *R. sinuata* are quite similar to each other but, as can be seen by comparing with Fig. 3, adaxial and abaxial epidermal walls of leaves of *R. sinuata* is apparently sinuate while *R. tenuifolia* is undulate (Fig. 4).

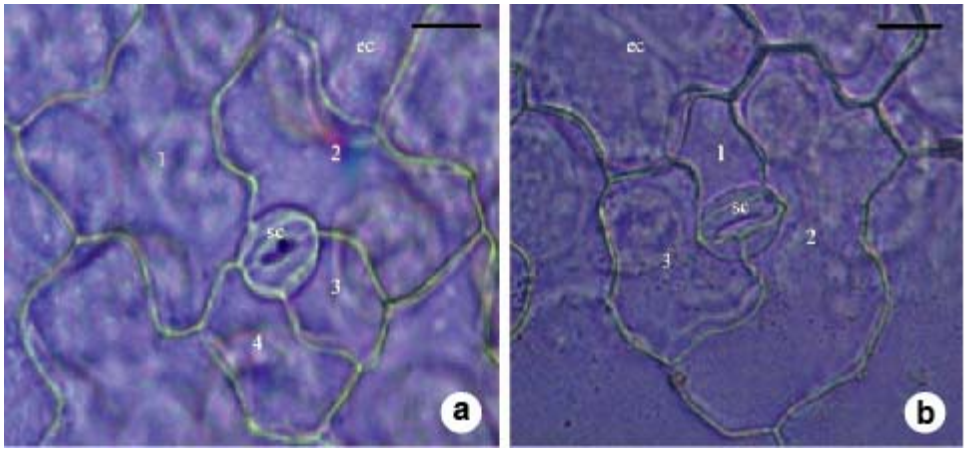

Fig. 5. Stomata types of *Ricotia* species. A. Anomocytic type, B. Anisocytic type. sc: stoma cell and ec: epidermis cell (Bars : 20 µm).

Based on the anatomical features of the leaf, leaf surface and stem, a diagnostic key is presented below.

1. Stem cortical parenchyma consists of 3 - 5 layers, interfascicular bundles are not clear; mesophyll type is bifacial; anticlinal walls of adaxial epidermal surface are straight or undulate.
   2. Pith region is narrow. Vascular bundles occupy the most space in stem. Phloem rises up to 11 layers in stem.
      3. Stem and leaf surface are glabrous; Epidermal surface of leaves are straight or undulate ***R. tenuifolia***
      3. Stem and leaf surface are sparsely haired (1 - 2 cells), Epidermal surface of leaves are sinuate ***R. sinuata***
   2. Pith region is wide. Vascular bundles occupy less space in stem. Phloem rises up to maximum 6 layers in stem ***R. carnosula***
1. Stem cortical parenchyma is 6 - 10 layered, interfascicular bundles are present; mesophyll type is equifacial; anticlinal walls of adaxial epidermal surface are all straight.

4. Stem and leaf surface denser, simple hair (1 - 4 cells); in leaf, sclerenchymatic tissue on the bundles is absent. Shape of epidermis cells of adaxial epidermal surface is polygonal ***R. davisiana***

4. Stem and leaf surface are glabrous or very sparsely haired. In leaf sclerenchymatic tissue on the bundles is present or absent. Shape of epidermal cells of adaxial epidermal surface polygonal

    5. Sclerenchyma on middle vascular bundle in leaf is absent. Pericycle is one layered and vascular bundles are not surrounded by pericycle ***R. varians***

    5. Sclerenchyma on middle vascular bundle in leaf is present. Pericycle is many layered and vascular bundles are surrounded by pericycle. ***R. aucheri***

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