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DETERMINATION OF AMINO ACIDS IN TWO SPECIES OF *RICCIA*

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ABSTRACT: Two species of *Riccia*, namely *Riccia billardieri* and *Riccia discolor*, were studied to detect various amino acids qualitatively as well as quantitatively through HPLC. Seven amino acids (histidine, serine, tryptophan, asparagine, tyrosine, valine, and isoleucine) were observed to be common in both the species but varying significantly in their quantities. *Riccia billardieri* contained additionally four (lysine, glycine, cystine, and proline) amino acids and *Riccia discolor* one (glutamine) amino acid. The contents of histidine (0.597 mg g^{-1}dw) and asparagine (0.323 mg g^{-1}dw) were found higher in *Riccia billardieri*, but the concentrations of tryptophan (0.927 mg g^{-1}dw), serine (0.169 mg g^{-1}dw), tyrosine (0.024 mg g^{-1}dw), valine (0.394 mg g^{-1}dw) and isoleucine (1.770 mg g^{-1}dw) were higher in *Riccia discolor*. The value of asparagine was almost similar in both the species. In *Riccia billardieri* the concentration of histidine (0.597 mg g^{-1}dw) was observed maximum, whereas in *Riccia discolor* isoleucine (1.770 mg g^{-1}dw) was found in the highest amount. The presence of amino acids indicates the potential use of *Riccia* in the pharmaceutical and food industry.

INTRODUCTION: Liverworts are known to have oil bodies, which consist of active chemical compounds. They produce several secondary metabolites such as diterpenoids, sesquiterpenoids and aromatic compounds [1]. Bryophytes consist of many biological compounds like sugars, oligosaccharides, polysaccharides, amino acids, alcohols, fatty acids, aliphatic compounds, aromatic and phenolic substances [2]. Traditionally, bryophytes were used to treat wounds, skin diseases, cuts and burns [3, 4]. Riccia is a genus of thallose liverworts in the order Marchantiales and the family Ricciaceae. It mostly grows in wet and damp terrestrial conditions. In the Himalayan region, species of *Riccia* were used to cure ringworm as indicated by its rosette forming growth habit resembling the rings made by the worm according to the Doctrine of Signatures. It can also be used effectively against the growth of bacteria or yeast [5]. The crude extracts of *R. nigerica* showed significant antimicrobial activity against both bacteria and fungi and have the potential to be used as an alternative to drugs [6]. Riccia can also be used to treat skin allergy, sores and lip cracks by applying its decoction with vegetable oil [7].

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Amino acids are the building blocks of the body and are needed by the human body as these acts as a precursor in many processes. Amino acids and peptides represent the basic fundamental structural units of proteins. In human body, amino acids are used in various biological processes like metabolism, transamination and decarboxylation.

Amino acids essential for human health are phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, histidine and lysine which must be supplied from outside as our body cannot synthesize them [8]. Conditionally essential amino acids arginine, cysteine, glycine, glutamine, tyrosine, and proline are useful components in various metabolisms, therefore, must be supplied exogenously in deficiency diseases. They are also required by the growing children and infants [9]. It is a well-known fact that deficiency of amino acids leads to fatigue, nausea, impaired antibody production, and weak immune system [10].

Recent studies on the seasonal variation in the content of free amino acids in three species of Marchantiaceae - *Marchantia nepalensis*, *M. palmata* and *Dumortiera hirsuta* [11], in three leafy liverworts – *Chiloscyphus gollani, Solenostoma crenulata* and *Fossombronia himalayensis* [12], in a thalloid liverwort *Targionia hypophylla* [13] and in a hornwort *Phaeoceros himalayensis* [14] revealed that their content was at peak in the rainy season and reduced to minimum in the winter season. This was an effect of maximum protein content due to lower activity of proteolytic enzyme protease. Devi *et al.*, [15, 16] studied the content of eight essential amino acids in the two species of *Marchantia* and in three species of *Plagiochasma* using HPTLC. 20 amino acids were investigated in *Targionia hypophylla* both qualitatively as well as quantitatively using the technique of HPLC [17].

To the best of our knowledge, no one had attempted to determine the content of amino acids in species of *Riccia* till date. The present study is aimed at investigating the presence or absence as well as the quantity of all the 20 amino acids in the two species of *Riccia* using the method of HPLC. This information can be useful in taxonomy, pharmaceutical, natural chemistry, and bio-molecular studies.

MATERIALS AND METHODS:

Collection and Preparation of Plant Material: Plant materials were collected from Kasauli and Mandi (Himachal Pradesh). The names of taxa, locality, altitude, nature of substratum, and herbarium reference numbers (PAN) are given in **Table 1**.

TABLE 1: *RICCIA* SPECIES DETAILS WITH HERBARIUM REFERENCE NUMBERS

Name of Taxon	Locality and altitude	Substratum	Herbarium reference number
Riccia billardieri Mont. et Nees	Mandi, 1044m	Wet soil on gravel in exposed habitat	PAN 6181
Riccia, discolor Lehm. et Lindenb.	Kasauli, 1927m	On moist soil in shady habitat	PAN 6182

The collected samples were carefully cleaned to remove soil and other adhering materials, washed with distilled water, separated, air dried, powdered, and used for further analysis. The methodology of HPLC, as reported by Thakur and Kapila [17], is followed.

RESULTS AND DISCUSSION: Of the eleven out of 20 studied amino acids, in *Riccia billardieri* five (lysine, histidine, tryptophan, valine, and isoleucine) are indispensable amino acids, four (tyrosine, cystine, proline and glycine) conditionally essential amino acids and two (serine and asparagine) are dispensable amino acids. **Fig. 1 (A-L)** shows the peaks of the standard of amino acids present in *R. billardieri* and *R. discolor*. The **Fig. 2** and **3** show the peaks of amino acids present in the samples of *R. billardieri* and *R. discolor*, respectively.

TABLE 2: AMOUNT OF VARIOUS AMINO ACIDS PRESENT IN *RICCIA BILLARDIERI* AND *RICCIA DISCOLOR* IN mg g^{-1}dw

Amino acids	*R. billardieri*	*R. discolor*
Serine	0.077	0.169
Isoleucine	0.005	1.770
Histidine	0.597	0.450
Valine	0.166	0.394
Tryptophan	0.300	0.927
Tyrosine	0.024	0.114
Asparagine	0.323	0.297
Lysine	0.205	-
Glycine	0.152	-
Glutamine	-	0.556
Cystine	0.420	-
Proline	0.124	-

In *R. discolor*, eight amino acids were detected out of 20 studied amino acids, of which four (isoleucine, valine, tryptophan, and histidine) are indispensable, two (glutamine and tyrosine) conditionally essential and two (serine and asparagine) are dispensable amino acids.

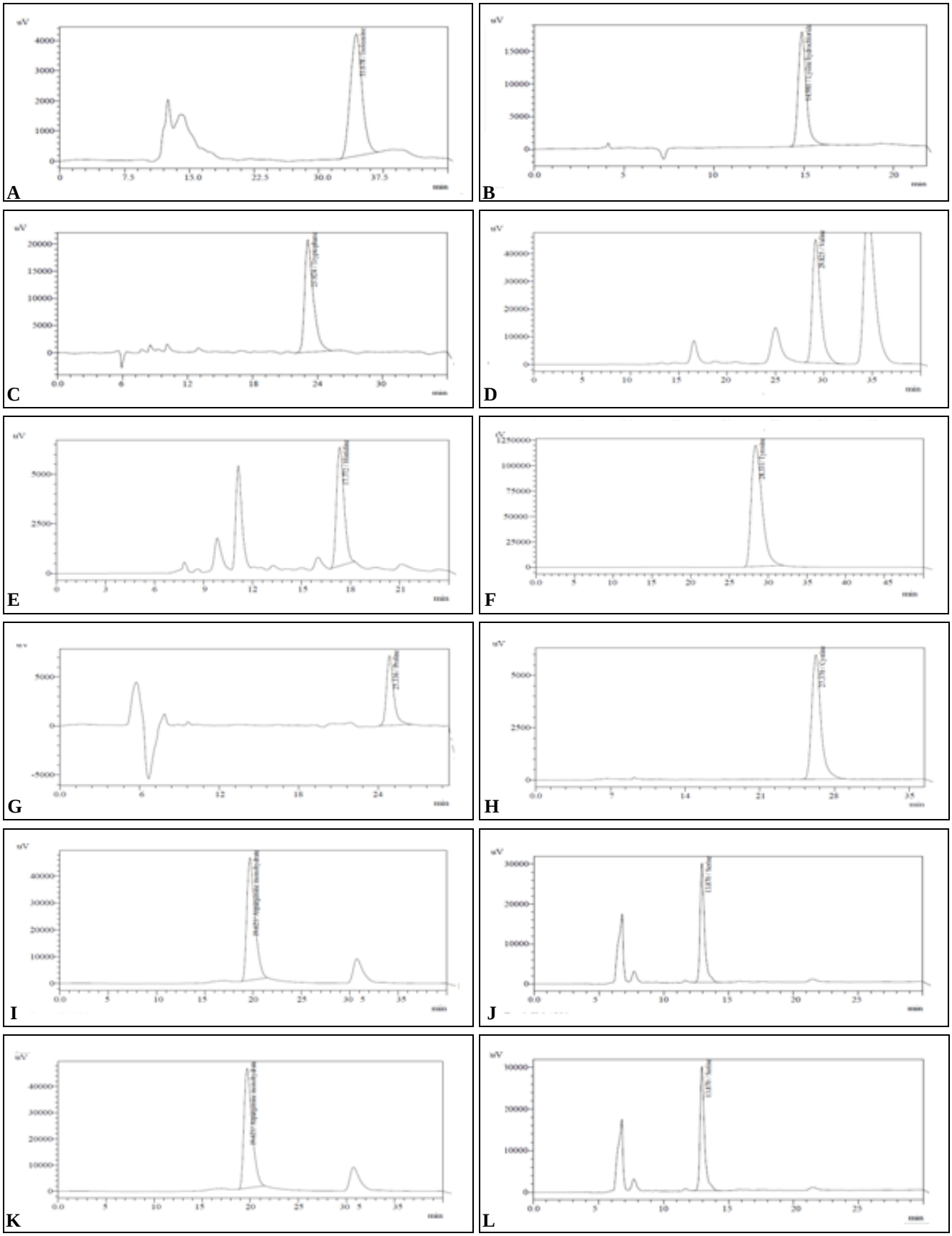

FIG. 1: CHROMATOGRAMS OF THE STANDARD AMINO ACIDS PRESENT IN *R. BILLARDIERI* AND *R. DISCOLOR i.e.* (A) ISOLEUCINE, (B) LYSINE, (C) TRYPTOPHAN, (D) VALINE, (E) HISTIDINE, (F) TYROSINE, (G) PROLINE, (H) CYSTINE, (I) GLYCINE, (J) GLUTAMINE, (K) ASPARAGINE AND (L) SERINE

Lysine, glycine, proline and cystine are found in *R. billardieri*, but they are absent in *R. discolor*, while glutamine is absent in *R. billardieri*. The respective contents of the amino acids present in two species are given in **Table 2**.

Of the amino acids present in both the species of *Riccia*, serine (0.169 mg g^{-1}dw), tryptophan (0.927 mg g^{-1}dw), tyrosine (0.114 mg g^{-1}dw), valine (0.394 mg g^{-1}dw) and isoleucine (1.770 mg g^{-1}dw) were detected in higher content in *R. discolor* as compared to *R. billardieri*, while the contents of histidine (0.597 mg g^{-1}dw) and asparagine (0.323 mg g^{-1}dw) were observed in higher quantity in *R. billardieri* than in *R. discolor*.

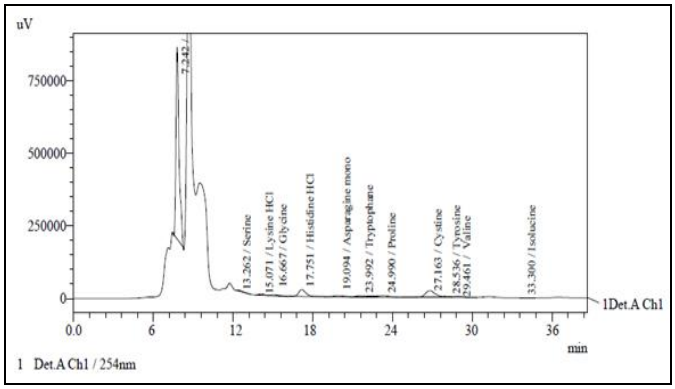

FIG. 2: HPLC CHROMATOGRAM OF *RICCIA BILLARDIERI*

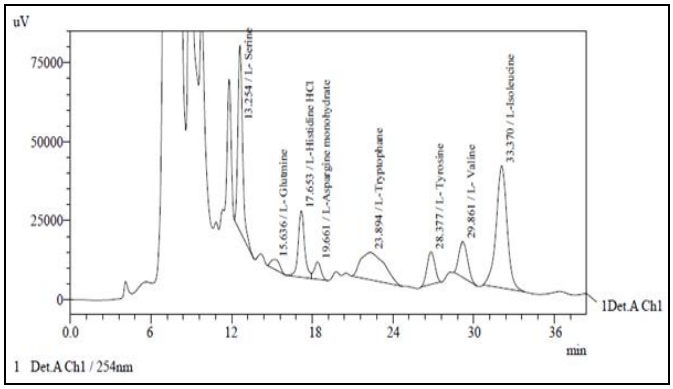

FIG. 3: HPLC CHROMATOGRAM OF *RICCIA DISCOLOR*

Serine is known to play a fundamental role in metabolism and signaling in living organisms and is also involved in plant response to biotic and abiotic stresses [18, 19]. Serine also helps in the biosynthesis of phospholipids, sphingolipids, nitrogenous bases, and amino acids required for cell proliferation [20]. It also boosts our immune system by enhancing the synthesis of antibodies. It reduces pain and is useful in treating mental illness. It is used as a natural moisturizing agent as well. Serine inhibits the cell growth of bacteria *Bacillus subtilis* and *Escherichia coli* [21, 22]. Due to the presence of serine, these plant species have the potential to be used in the cosmetic and pharmaceutical industry and also as a food preservative for inhibiting the growth of bacteria.

Isoleucine is one of the branched chain amino acids that is required as a substrate for the protein synthesis in all the organisms and also required for the cell proliferation and expansion during root development [23]. In plants, deficiency of isoleucine affects the hormone jasmonate signaling by decreasing the formation of the jasmonate-isoleucine conjugate, leading to insect attack susceptibility and also impairing the root development [24, 25, 23]. Presence of this amino acid in both the species might be responsible for the resistance of these species to the insect attack and its antimicrobial activity. It can be used for treating skin infections caused by bacteria and fungi.

Devi *et al.*, [15, 16] investigated the content of amino acids in the two species of *Marchantia* and three species of *Plagiochasma* using the technique of HPTLC. Among the three species of *Plagiochasma*, isoleucine was not observed in *P. appendiculatum*, while present in *P. intermedium* and *P. articulated* as well as both the species of *Marchantia nepalensis and M. palmata.*

Histidine plays a significant role during plant growth, reproduction, seed development, amino acid transport, and source-sink relationship and it is also one of the indispensable amino acids in proteins and also acts as a metal-binding ligand[26, 27]. Presently, the content of histidine was found higher (0.450 - 0.597) than the previous reports made by Devi *et al.*, [15, 16] in two species of *Marchantia* (0.254 - 0.279) and three species of *Plagiochasma* (0.066 - 0.424).

Valine is one of the three branched chain amino acids and plays a pivotal role in protein synthesis and in the plant as well as animal growth [28, 29]. It also acts as a precursor for secondary metabolites like acyl-sugars, glucosinolates, and cyanogenic glycosides. Valine is also needed for tissue repair, muscle metabolism, and the maintenance of proper nitrogen balance in the body. Accumulation of valine in crop plants significantly increases the nutritional value of food as well as feed [30]. Presence of histidine and valine in these species

helps in the growth and development of plant body and is also indicative of their potential to be used in the food industry for making nutrient supplements. The content of valine reported by Devi *et al.,* [15, 16] in the two species of *Marchantia* (0.031 - 0.53) and three species of *Plagiochasma* (0.031 - 0.340) was lower than the presently observed content in two species of *Riccia* (0.166 - 0.394). Amino acid tryptophan acts as a precursor of secondary metabolites such as indolicphytoalexins, terpenoidindole alkaloids, indoleglucosinolates and indole-3-acetic acid (IAA) in plants [31, 32]. Plant hormone indole-3-acetic acid (IAA) plays a pivotal role in apical dominance, tropisms, cell expansion and embryo development [32, 33]. Tryptophan also plays a significant role in defense responses. The stress conditions induce the synthesis of tryptophan, which in turn regulates the development of plant [34, 35]. Tryptophan, an essential amino acid, is used to synthesize serotonin (a mood-determining brain chemical) and also cannot be produced without serine [36]. Shortage of tryptophan and serotonin causes depression, insomnia, and anxiety. Presence of tryptophan in both the presently studied species of *Riccia* might be helping in the growth and development of plants in exposed conditions and also suggests their use in the pharmaceutical industry.

Tyrosine is a proteinogenic amino acid which plays a pivotal role in the signal transduction processes as a receiver of phosphate group [37]. It is also known to play a significant role in photosynthesis. Tyrosine kinases and phosphatases are known to play a significant role in the regulation of growth and differentiation of cells in animals [38]. Phosphorylation of tyrosine during various processes in plants is known to regulate various developmental, biotic, and abiotic responses [39]. It is useful to relieve stress, fatigue, and depression. It strengthens our immune system and increases the production of the neurotransmitter, melamine in the body. Tyrosine supports neurotransmitters in the brain, and also helps to reduce stress, acts as an appetite suppressant, and improves mental alertness.

Presence of tyrosine in the presently studied species is suggestive of its role in the plant growth and development as well as in their survival during stress conditions. Due to the presence of tyrosine, these plant species also have the potential to be used in the pharmaceutical industry. Asparagine is a key amino acid for nitrogen storage and transport in plants. It is an important amino acid in plants because it has a high ratio of N/C compared to other amino acids, making it ideal nitrogen storage and transport compound [40]. About this feature, many important biological functions in plants have been ascribed to asparagine such as nitrogen recycling during abiotic and biotic stresses or nitrogen mobilization and transport from source to sink tissues [40, 41]. The presence of asparagine might be responsible for the growth of presently studied species in exposed habitats and also suggest their use in the pharmaceutical industry for making drugs.

The degradation of lysine plays a pivotal role in the proper functioning of the mammalian brain by synthesizing glutamate, which regulates signals of nerve transmission through glutamate receptors [42]. In response to various environmental changes and stress conditions, lysine catabolizes into glutamate and affects the growth, development, and other physiological processes in the plant body [43]. Presence of lysine in *R. billardieri* helps this species to grow successfully in exposed and brightly lit conditions. During the synthesis of a variety of chemicals, glycine acts as an intermediate, and it can also be used for the formation of glyphosate - an herbicide [15]. The presence of glycine in *R. billardieri* suggests that these plants can be used as an alternative source for the manufacture of this herbicide.

Glutamine synthetase helps in the metabolism of nitrogen, and it is the most important component of NUE (Nitrogen Use Efficiency) as well as plant yield [44, 45]. Ammonia produced during nitrogen fixation is assimilated by the glutamine synthetase which is also known for its role in nitrate or ammonia nutrition since the activity of enzyme glutamine synthetase is positively correlated with nitrate content, and it also promotes leaf senescence [46]. Glutamine is converted to glucose in case the human body needs more glucose as an energy source; it is also important in ATP production [47]. So, the presence of glutamine in *R. discolor* is responsible for nitrogen metabolism in this plant and provides nutrition and energy to the plant for its growth and development.

Cystine proteinases play a pivotal role in storage, accumulation as well as metabolization of proteins, in plant growth, development, senescence, and signaling during environmental disturbances [48]. They play a key role as a signaling molecule for regulating various processes in the plant like photosynthesis, plant defense against stressed conditions, plant immune responses, autophagy, and the development of the roots [49]. Cystine present in both the presently studied species must be helping in the growth and development of plant and also regulating defense responses. Proline plays a significant role in the plants growing in a stressful environment. During stress, it acts an antioxidant, signaling molecule, metal chelator as well as an excellent osmolyte [50]. During reproduction, proline acts as a source of energy to sustain this metabolically demanding process of the plant life cycle and also provides protection from osmotic damage during dehydration prone processes such as pollen development and embryogenesis [51]. Proline also plays a significant role in the maintenance of blood pressure and prevention of arteriosclerosis. Therefore, the presence of proline in these species helps them to survive in adverse environmental conditions and also to protect them from osmotic damage.

CONCLUSION: The species of *Riccia* showed the presence of important amino acids, which were identified and quantified for the first time using HPLC technique. *R. billardieri* showed the presence of 11 amino acids whereas, in *R. discolor* eight amino acids were present. Between the two species of *Riccia*, *R. discolor* showed higher content of various amino acids than *R. billardieri*. The presence of proline and lysine supported the growth and survival of *R. billardieri* in exposed condition.

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