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Riccia atromarginata (Ricciaceae, Marchantiophyta) new to the Western Balkans, with notes on its morphology

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Ključne besede: mahovi, jetrenjaki, steljka, spore, primerjalna analiza, Podgorica, Črna gora.

Abstract

This is the first record of the liverwort *Riccia atromarginata* for the Western Balkans. It was found on conglomerate terraces in the city of Podgorica, Montenegro, at the confluence of the rivers Ribnica and Morača. The material from Montenegro as well as living samples from Tenerife, Gran Canaria and Cyprus served as a basis for a detailed description of the main characteristics of the species, on the basis of which *Riccia atromarginata* can be reliably identified. However, for some characteristics there is a clear variability.

Izvleček

V prispevku poročamo o prvem nahajališču jetrenjaka *Riccia atromarginata* na zahodnem delu Balkanskega polotoka. Nahajališče se nahaja na konglomeratnih terasah sotočja rek Ribnice in Morače v Podgorici (Črna gora). Za natančen opis glavnih značilnosti vrste smo uporabili primerke iz Črne gore ter z otokov Tenerife, Gran Canaria in Ciper Za nekatere značilnosti vrste smo ugotovili jasno variabilnost.

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Introduction

The thallous liverwort *Riccia atromarginata* Levier has a Holarctic distribution. It grows from the Mediterranean to the Arab peninsula, central Asia, and in North and Central America (Jovet-Ast 1986, Schuster 1992, Bischler 2004). According to Bischler (2004), in the Mediterranean, the species is distributed across Portugal, Spain, Algeria, Tunisia, Libya, Egypt, Israel, the Canary and Balearic Islands, Morocco, France, Italy, Sardinia, Sicily, Greece, and Crete. *R. atromarginata* was also reported in Turkey (Özenoğlu et al. 2019). The recently published checklist of European Bryophytes (Hodgetts & Lockhart 2020) lists additional records from Madeira and Cyprus in accordance with Sérgio et al. (2006) and Frahm et al. (2009).

Dierßen (2001) described the range of the liverwort *R. atromarginata* by vegetation zones – occurring in tropical and Mediterranean regions with evergreen hardwood and coniferous forests, as well as meridional steppes and semi-deserts in the continental parts. It grows on dry, moderately acidic to basic, compact soils with low to medium nutrient content, fully exposed to light and often wind. Habitats range from dune sand with some trampling influence, to deep or thin open soil between woody plants, stones and rocks.

Study area

Podgorica, the capital city of Montenegro, is located in the south-eastern part of the country. The largest part of Podgorica-Skodra valley, as well as the northwestern, northern and eastern parts of the surrounding mountain slopes (Kučke and Piperske mountains) administratively belong to Podgorica, as do the hills of the Lješanska nahija (Radojičić 1996; Radojičić 2002). The hydrological network consists of the Morača River and its tributaries: Zeta, Cijevna, Ribnica, Matica, and Sitnica, as well as the Mareza springs (Ičević 2003). Geologically, the area is characterized by Quaternary sediments, represented by glaciofluvial, alluvial, terrace and lake sediments. (Đokić & Petrović 1974). The calcareous alluvial deposits of gravel and sand turned into the conglomerate on which a shallow and skeletal layer of brown eutric soil was formed (Fuštić 1997; Fuštić & Đuretić 2000). The area of Podgorica belongs to the thermophytic deciduous forest vegetation zone, characterized mainly by the association *Rusco-Carpinetum orientalis* (Blečić & Lakušić 1976). Only small fragments of natural vegetation are present (on the city's hills: Gorica, Malo Brdo, Dajbabska Gora and Kakaricka Gora, (Stešević & Jovanović 2008), since it is replaced by secondary vegetation comprised of grasslands, agricultural fields, urban areas, etc. According to the Köppen climate classification (Köppen 1936), Podgorica and the surroundings belong to the climate subtype Csa – Mediterranean climate characterized by hot, dry and clear summers, and mild and rainy winters; while summer is the driest period of year, maximum rainfall occurs in November, and is lowest in July. The climate is different from the real Mediterranean climate because of its slightly colder winters (Burić et al. 2014). According to Burić et al. (2012), the mean annual precipitation in Podgorica is 1654 mm, occurring mainly during the winter. There are 121 rainy days and 129 summer days with mean temperature above 24 °C. The mean annual temperature is 15.3 °C, the mean highest temperature is 26.4 °C in summer and 7.6 °C in winter. Snowfall is very rare.

The area Skaline, were the Ribnica River flows into the Morača River, is a frequently visited historic place in the city centre of Podgorica. During the Holocene, the rivers incised deep erosion valleys into the fluviatile conglomerate. The cliffs are steep, and often vertical.

Methods

Our work was based on descriptions of the main morphological characters of the *R. atromarginata* from Podgorica and our living samples from Tenerife, Gran Canaria and Cyprus, and its comparison with available data from the literature: the shape and width of the thallus, margins, sides, groove, ventral scales, cilia, and spores. To do so, we measured a thalli, and made cross sections of our material, and inspected and measured spores under a light microscope. We tested various agents commonly used in microscopy for bleaching *Riccia* spores because of its very dark spores which not allow a clear view of all spore characteristics (for example the wall, which is important for determination). Bleaching with chloral hydrate only sometimes worked. Otherwise, we used sodium hypochlorite, which leads to completely hyaline spores within a couple of minutes (Berg & Pöltl 2020b).

Results

Riccia atromarginata, a new species for the bryophyte flora of Montenegro

Riccia atromarginata Levier was found in Skaline, at the confluence where the river Ribnica flows into the river Morača, in Podgorica (Figure 1). Its habitat is in shallow soil on conglomerate terrace along the rivers, with southern exposure (N 42°26'22.49" E 19°15'30.06", 22 m a.s.l., leg. S. Dragićević & Ch. Berg, 27.02.2020, GJO 101207, *Riccia* project-ID ABry_517).

Figure 1: Skaline, at the confluence of the Ribnica and Morača rivers, the location where *Riccia atromarginata* was discovered.
Slika 1: Skaline, sotočje reke Ribnice z reko Moračo, nahajališče vrste *Riccia atromarginata*.

Figure 2: Habitat of liverwort *Riccia atromarginata* on Skaline in Podgorica.
Slika 2: Habitat vrste *Riccia atromarginata* na območju Skaline v Podgorici.

These terraces are often covered by city buildings or parks, or are used as pastures (sometimes only sporadically). Nearby terraces with deeper soil layers are covered by more mesophilous grasslands, while in areas with a thin soil layer, which are more suitable for liverworts, dry grassland dominates (Figure 2). The conglomerate terraces of the river Morača in Podgorica provide an extraordinary habitat for thallose liverworts, where besides *R. atromarginata*, *Reboulia hemisphaerica, Mannia androgyna, Oxymitra paleacea, Riccia nigrella, R. ciliata, R. papillosa, R. michelii* and some not yet identified *Riccia* species occur. Inside this habitat type, *R. atromarginata* prefers patches of open soil at or near the upper cliff edge, where grassland vegetation is even sparser due to erosion processes.

Morphology of the species

Descriptions of *Riccia atromarginata* were given in the protologue of Levier (1889) and later Müller (1906–1911), Jovet-Ast (1986), and Schuster (1992). For the description presented here, we use primary data from our "*Riccia* morphology and sequencing project", which include collections of *R. atromarginata* from Tenerife (GJO 101208), Gran Canaria (GJO 100874), Montenegro (GJO 101207) and Cyprus (GJO 101206, GJO 101209).

Riccia atromarginata belongs to the larger *Riccia* species of the subgenus *Euriccia*, with thallus segment width often exceeding 1 mm. In our samples, thalli width is (0.4–)0.8–1.7(–1.8) mm. The thallus segments are ovate

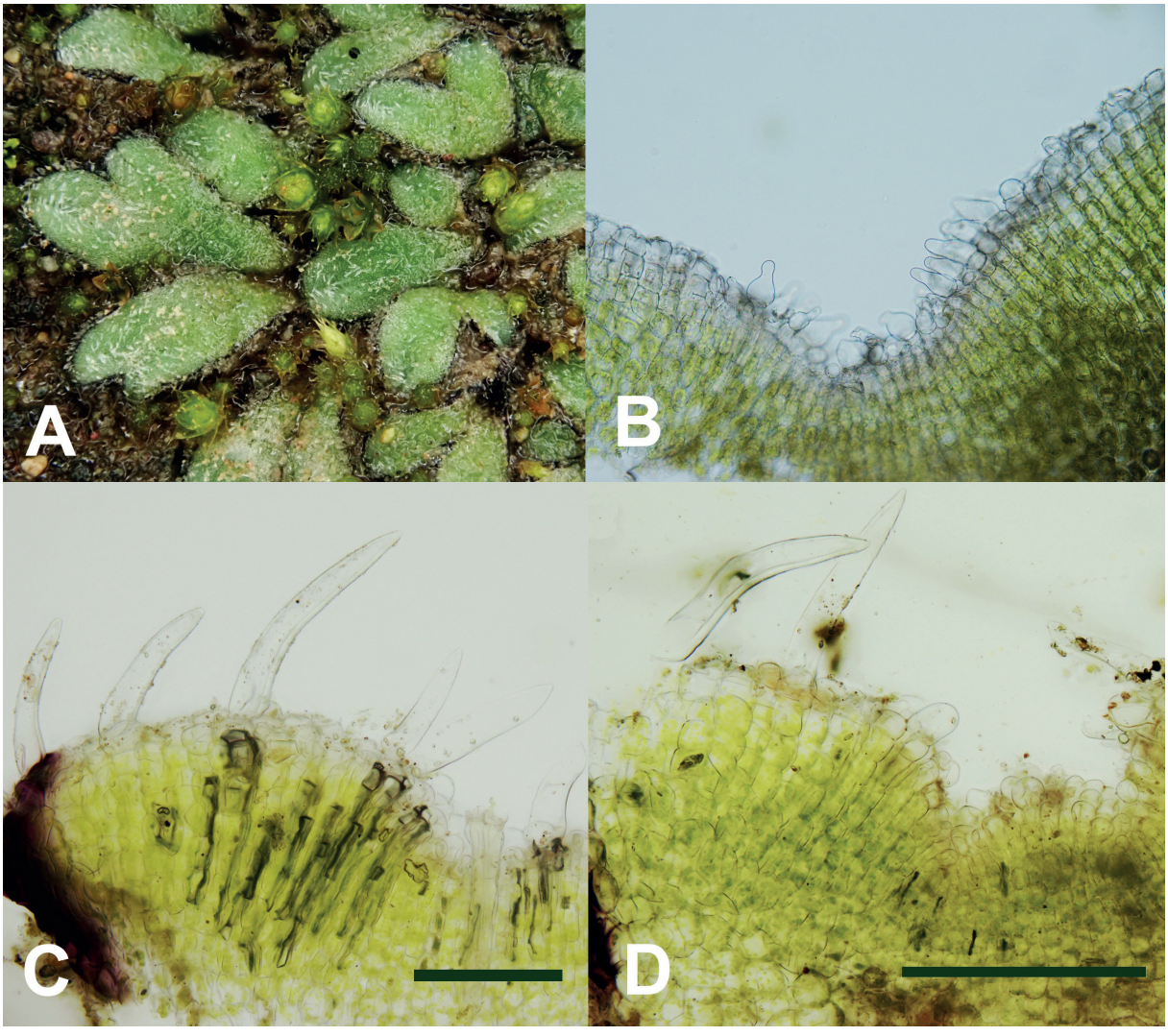

Figure 3: *Riccia atromarginata* **A** Thalli from Gran Canaria with numerous cilia even on ventral thallus side; **B** cross section of thallus from Montenegro with epidermal cells of different shape and size; **C, D** cross section of ultimate thallus segment with dorsal cilia, collection from Tenerife. Scale bar 200 µm.
Slika 3: *Riccia atromarginata* **A** steljka s Kanarskih otokov s številnimi cilijami tudi na ventralnem delu; **B** prerez steljke iz Črne gore s celicami epidermide različnih oblik in velikosti; **C**, **D** prerez sprednjega dela steljka z dorzalnimi cilijami; zbirka s Tenerifa. Merilo 200 µm.

to oblong with rounded apices growing in loose batches or small groups, but not in rosettes, sometimes as undivided segments, often with one or two furcations, rarely with more. The upper side is vivid green in young parts, and dark bluish green in older ones. Margins and sides are conspicuous deep purple, visible from dorsal view. The groove is sharp and deep on the thallus apex but opens just behind the top becoming wide and flat on the terminal thallus segments, while older thallus parts are mostly flat or even convex on the dorsal surface. Ventral half scales at the thallus apex are small, deep purple to hyaline and disappear towards thallus centre. The marginal thallus edge is sharp to obtuse. On this edge, sometimes even on the upper surface and the sides, conic, obtuse to acute, hyaline cilia (in some determination keys called "papillae") are formed with a length up to 270 µm (Figure 3A, C and D). At the ultimate segment, short conical cilia nearly always exist at the marginal edges. They are bristle-like, and stand dense together in two or more rows forming a fringe (Figure 3C). The epidermal cells are normally globose or pyriform with high variability (Figure 3B). The appearance and length of cilia varies greatly within the distribution range, and even within one population, where thalli with many cilia can occur right next to nearly glabrous ones. All epidermal cells including enlarged ones erode away with age. In cross section, the youngest thallus-segments are (1)2–3 times wider than high.

In contrast to other descriptions in the literature and our living samples from the Canary Islands and Cyprus, the thalli from Podgorica do not show cilia on the dorsal surface, and the marginal cilia are comparable short (up to 50 µm). The marginal edges are rather sharp in cross section (Figure 4).

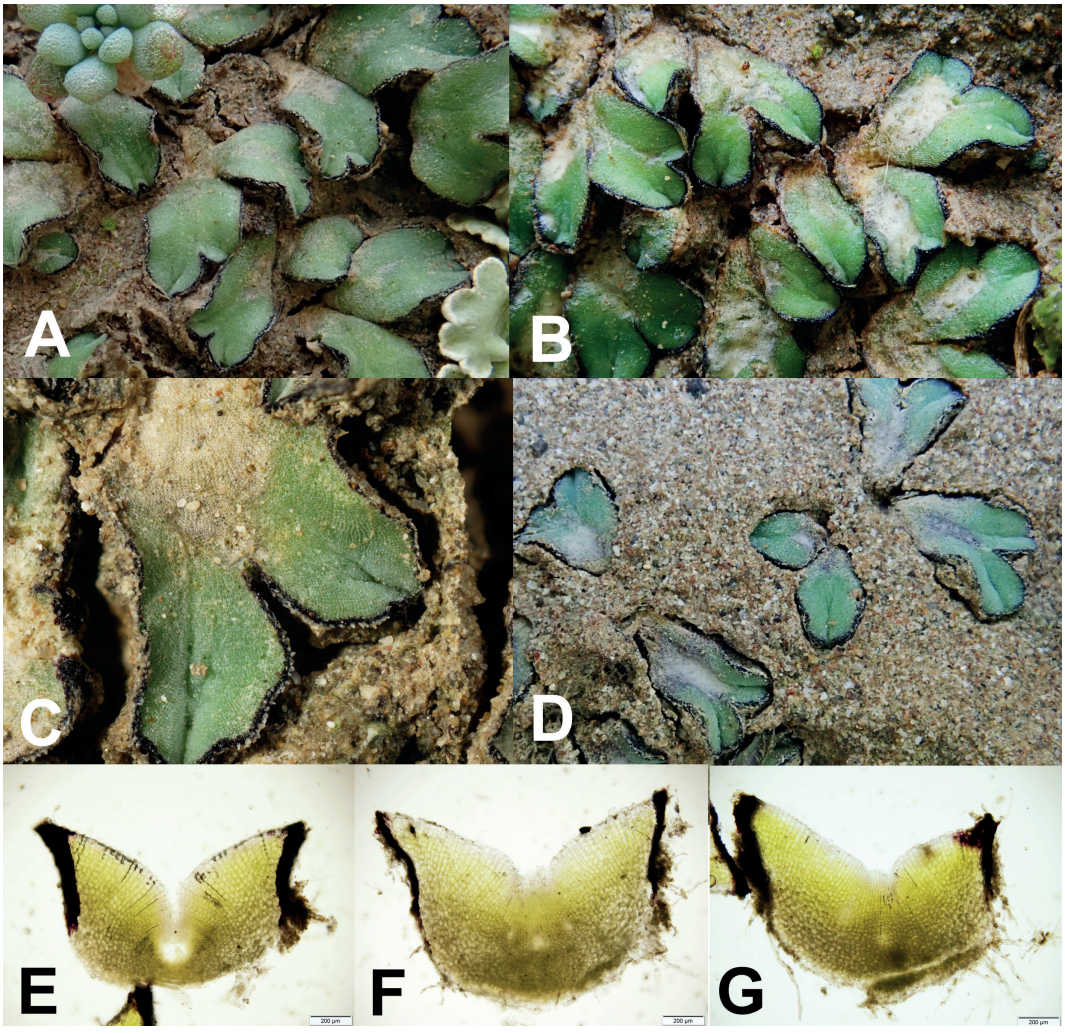

Figure 4: *Riccia atromarginata* from Podgorica, **A–D** habitus view and **E–G** cross section of ultimate thallus segment.
Slika 4: *Riccia atromarginata* iz Podgorice, **A–D** habitus, **E–G** prečni prerez sprednjega dela steljke.

The species is considered as dioecious by Jovet-Ast (1986). Levier (1889) wrote "planta ut videtur dioica" means "looks like dioicous". Schuster (1992) considered the species as monoicous with the remark "sometimes appearing dioicous!". This feature is not easy to verify. In our living collection, *R. atromarginata* was sporulate in all our samples, while dioicous species like *Riccia cilliifera*-complex or *R. michelii* remain without capsules over more than two years of cultivation.

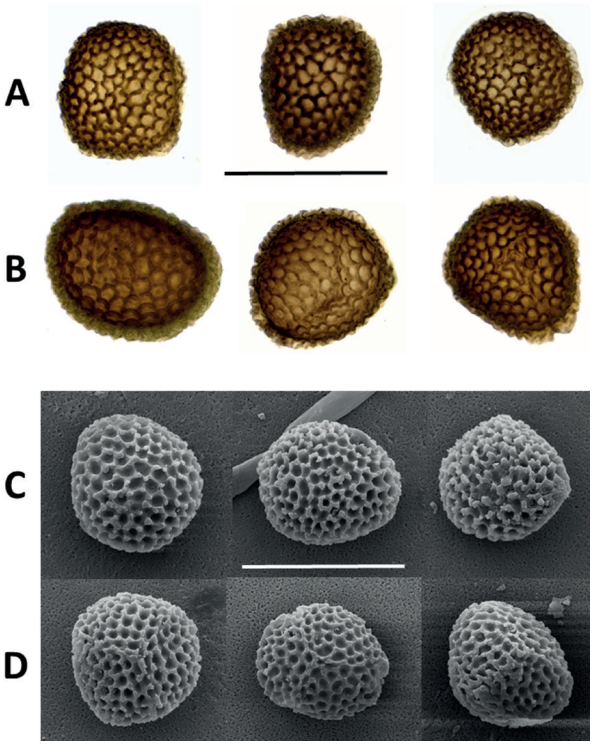

Figure 5: Spores of *Riccia atromarginata* from Podgorica, **A** distal view light microscope, **B** proximal view light microscope, **C** distal view scanning electron microscope, **D** proximal view scanning electron microscope. Scale bar 100 µm.

Slika 5: Spore vrste *Riccia atromarginata* iz Podgorice. **A** distalno (svetlobni mikroskop), **B** proksimalno (svetlobni mikroskop), **C** distalno (elektronski mikroskop), **D** proksimalno (elektronski mikroskop). Merilo 100 µm.

The spores are rather characteristic, with a somewhat apolar appearance, i.e., spores look similar from every side view, without a pronounced proximal and distal face (Figure 5). Spores are more globose than round triangular like in typical polar spores (f.e. *R. glauca* or *R. bifurca*, see Berg & Pöltl 2020a). The marginal wing is nearly lacking or small, and under a light microscope, a light margin can appear due to the loose exospore (Figure 5B), but the wing is never typical smooth, and never without ornamentation. The distal face of the spores shows 10–20 areoles in diameter, with nearly the same sized areoles all around the spore. Proximal triradiate ridges are only weakly indicated, but a weak edge is visible giving an idea of proximal triangles (Figure 5B, 5D). In all *Riccia*-literature, spore size is considered as a stable and diagnostic feature. Within all analysed *R. atromarginata*-samples, the spores from those of Podgorica are comparably small with 64 µm minimum, 90 µm mean, and 116 µm maximum size; 93% of all spores are 80 µm or larger (54 spores measured). Our samplings from Tenerife, Montenegro and Cyprus differ significantly in spore size, with a majority of spores larger than 80 µm and a mean diameter around 100 µm (Figure 6). We measured 140 spores so far from three individuals of three different population from Tenerife, Montenegro and Cyprus, with minimum size of 64 µm, lower quartile 91 µm, median 101, mean 106 µm, upper quartile 120 µm and maximum 159 µm.

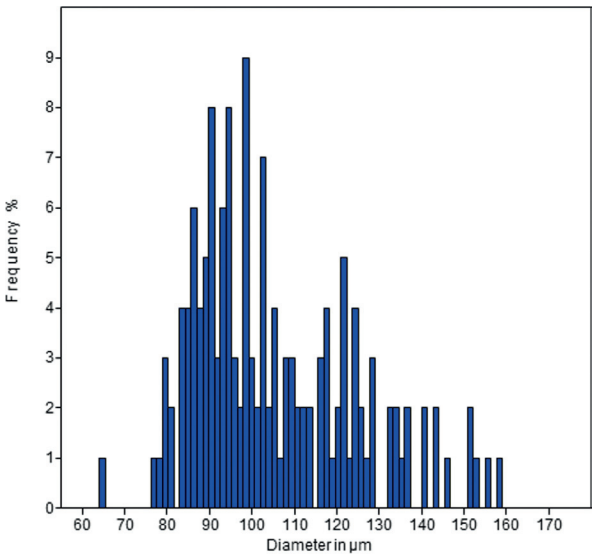

Figure 6: Distribution of spore diameter (in µm) of *Riccia atromarginata* based on 140 spores from three individuals from Tenerife, Montenegro and Cyprus.

Slika 6: Premer spor vrste *Riccia atromarginata* na osnovi meritev 140 primerkov s Tenerifa, Cipra in Črne gore.

The histogram of this measurements (Figure 6) shows a striking bimodal distribution. This could indicate (a) a high variability of spore size between populations or between capsules, or (b) the genetic influence of *R. trabutiana* (or possibly another cryptic species) with much smaller spores, or (c) a continuum of characteristics between both taxa. Mature spores are dark brown to black, and are difficult to observed under a light microscope.

Discussion

Even in light of the taxonomic uncertainties, we consider our sample to be *Riccia atromarginata*, because under the current state of knowledge, the sum of characteristics of thallus and spores points clearly in the direction of this species. Schuster (1992) discussed the high global variability of the species, but concluded that the common characteristics were so striking that it nevertheless counted as a unique species. However, uncertainty always remains on a global scale, especially because there are similar species outside the known area of *R. atromarginata*, like the Australian *R. inflexa* Taylor, whose identity has not yet been finally clarified (Cargill et al. 2016).

The example that we present here shows that many problems are still unsolved in this genus. In our "*Riccia* morphology and sequencing project" (Berg & Pöltl 2020a, 2020b) we try to detangle the European taxa using morphological and molecular data, and with the help of living collections. Many species, like *Riccia subbifurca* or *R. ligula*, are unclear, and many species pairs (e.g. *R. ciliata/intumescens*, *R. ciliifera/gougetiana, R. commutata/warnstorfii, R. beyrichiana/michelii*) need deeper analysis of their delimitation. However, *R. atromarginata* seems to be one of the more easily identifiable taxa; only the delimitation to *R. trabutiana* could be problematic. This taxon is similar with dark red margins and spores of similar structure, but smaller in all parts including spore size, and never seen with papillae or ciliae, which are characteristic for *R. atromarginata*.

This new record is important as it helps us in our aim to resolve morphological differences between European *Riccia* taxa, but also fills the distribution gap between Italy and Greece.

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