The morphologically cryptic lichen species *Parmelia* ernstiae and *P. serrana* new to Norway

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The two species *Parmelia ernstiae* and *P. serrana* are reported as new to Norway from two collections each, identified by the DNA barcode marker (nrITS). A chemical analysis of selected specimens of *P. saxatilis* s. lat. revealed an additional 29 collections of the two species due to the presence of fatty acids. An analysis of seven morphological and chemical characters currently used for distinguishing the two species failed, however, and the two species are hence regarded as morphologically cryptic.

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Introduction

Parmelia saxatilis (L.) Ach. is a common foliose lichen species in the Nordic countries. Holien (1998) recognized two enteties of the species in Central Norway based on the correlation of morphological characters (lobe width, position of the isidia, and size of the rhizines) with a chemical character (presence/absence of fatty acids). Subsequent molecular studies showed that P. saxatilis is heterogeneous in its classical concept, and the two newly described species P. ernstiae Feuerer & A. Thell and P. serrana A. Crespo et al. were reported to occur in our area by Thell (2003) and Mattsson et al. (2006), respectively. Both species grow primarily on bark of deciduous trees and are currently known from Denmark, southern Sweden, and southern Finland (Thell et al. 2011, Thell et al. 2017, Nordin et al. 2019). Parmelia saxatilis s. str., which is ubiquitous in the Nordic area, is most common on rock except for in Denmark and coastal Norway where the corticolous habitat dominates (Thell et al. 2011).

The most recent and comprehensive taxonomic study of the *P. saxatilis* complex in Europe was conducted by Ossowska et al. (2018), using the fungal DNA barcode marker (nrITS) for the phylogenetic analysis. They found atranorin and salazinic acid to be always present in *P. ernstiae*, *P. saxatilis*, and *P. serrana*; lobaric acid occurred in all three species, although variably, but fatty acids (mainly lichesterinic and protolichesterinic acids) were restricted to *P. ernstiae* and *P. serrana*. Fatty acids occurred in all examined specimens of those two and were hence regarded as a diagnostic character for the separation of the two species against *P. saxatilis*. Ossowska et al. (2018) also showed that all three species can develop epruinose to strongly pruinose thalli and concluded that pruinosity is "probably insignificant in the taxonomy of the genus". This leaves the morphological identification of the three species to more or less subtle differences in the shape, size, and degree of overlapping of the lobes, in the presence of marginal lobules, and in the position of the isidia on the lobes and thallus. Phylogenetically, *P. saxatilis* formed a highly

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supported clade, but the two other species each formed unsupported sister clades and a combined unsupported clade in Ossowska et al. (2018).

Parmelia ernstiae and P. serrana are not known from Norway (Nordin et al. 2019), although the presence of the latter in Nord-Trøndelag was indicated by Thell et al. (2017) by reference to the data portal Virtuella Herbariet (http://herbarium.emg.umu.se/databases.html). The only P. serrana from Norway in that database (accessioned 2019-02-10) is F278174 (S), but it is not clear if this specimen was identified by TLC and/or DNA data. We actually joined the excursion on which the specimen was collected and collected P. ernstiae (identified by DNA, see below) on the same locality, and hence regard the identification of F278174 as doubtful.

During a project on production of DNA barcode reference sequences for Norwegian lichens under the Norwegian Barcode of Life project (NorBOL, http://www.norbol.no), we have sequenced six specimens of *P. saxatilis* s. lat., and found two of them (from one locality) to belong to *P. ernstiae*, two to *P. serrana*, and two to *P. saxatilis* s. str. Subsequent TLC examination of selected, mainly corticolous Norwegian collections of *P. saxatilis* s. lat. from most of the Norwegian counties in O revealed 29 additional specimens containing fatty acids (i.e., either *P. ernstiae* or *P. serrana* in the circumscription of Ossowska et al. 2018). We attempted to identify 26 of those specimens, plus the four specimens identified as *P. ernstiae* and *P. serrana* from the DNA sequences, by scoring the morphological characters emphasized as diagnostic by Thell et al. (2017) and Ossowksa et al. (2018).

Material and Methods

The six specimens were sequenced and edited through the pipeline at the Canadian Centre for DNA Barcoding (CCDB; http://www.ccdb.ca), using the primer pairs ITS1-F/ITS4 or ITS5/ITS4. In addition, 21 sequences were downloaded from GenBank (Table 1) among which 19 were selected as to represent the morphological and geographical variation within the species of the *P. saxatilis* group (*P. ernstiae*, *P. mayi*, *P. omphalodes*, *P. pinnatifida*, *P. saxatilis*, *P. serrana*, and *P. submontana*) in the phylogeny of Ossowska et al. (2018). The remaining sequences were one of *P. ernstiae* from Sweden published by Thell et al. (2008) and one of *P. sulcata* used as the outgroup.

The dataset was edited in BioEdit v. 7.2.5 (Hall 1999) and aligned by the bundled software ClustalW (Thompson et al. 1994). The editing was limited to trimming ends and inserting one gap. The alignment was analysed by the maximum likelihood (ML) method by RAxML v. 8.2.10 (Stamatakis 2014) using the "ML + rapid bootstrap" method with 100 bootstrap repeats and the GTRGAMMA substitution model. The best-scoring ML tree was edited in TreeGraph2 (Stöver & Müller 2010).

All specimens were examined by thin-layer chromatography (TLC), performed in accordance with the methods of Culberson (1972) as modified by Menlove (1974) and Culberson & Johnson (1982), in solvent system B' on glass plates.

Seven chemical and morphological characters were scored in 30 specimens identified by TLC as being either *P. ernstiae* or *P. serrana*: (1) Lobaric acid present, (2) largest lobe width, (3) lobules present, (4) isidia marginal on the lobes, (5) isidia mainly in the thallus centre, (6) isidia on ridges, and (7) pruina present. Characters 1–5 are diagnostic according to Ossowska et al. (2018), and 6–7 are additional characters used by Thell et al. (2017). The presence of overlapping lobes was not included as we found it difficult to evaluate. The scores were normalized so that a value of +1 indicated *P. ernstiae*, –1 indicated *P. serrana*, and 0 was indecisive. The specimens were then attempted identified by the sum of scores being positive or negative.

Table 1. DNA sequences (nrITS) used in this study with voucher information and GenBank accession numbers. Sequences provided for this work are in bold.

Species	Country	Voucher ID	GenBank ID
P. ernstiae	Denmark	LD 1062891	EF406114
P. ernstiae	Germany	HBG 4619, holotype	AF410833
P. ernstiae	Latvia	UGDA L-19917	KU845673
P. ernstiae	Norway	O L-201277	MK567162
P. ernstiae	Norway	O L-201279	MK567160
P. ernstiae	Spain	MAF 9749	AY295110
P. ernstiae	Sweden	LD 1026466	AY247007
P. ernstiae	UK	MAF 6886	AF350041
P. mayi	USA	MAF 15765	JN609439
P. omphalodes	Spain	MAF 6055	AF350046
P. omphalodes	Spain	MAF 7062	AY036998
P. pinnatifida	Russia	MAF 7272	AY036988
P. saxatilis	Czech Republic	UGDA L-21201	KU845666
P. saxatilis	Norway	O L-184724	MK567164
P. saxatilis	Norway	O L-195994	MK567163
P. saxatilis	Sweden	MAF 6882, epitype	AF350028
P. serrana	Czech Republic	UGDA L-21196	KU845660
P. serrana	Norway	O L-195995	MK567161
P. serrana	Norway	O L-205007	MK567159
P. serrana	Poland	UGDA L-21211	KU845671
P. serrana	Poland	UGDA L-21227	KU845678
P. serrana	Spain	MAF 6885	AF350040
P. serrana	Spain	MAF 9756, holotype	AY295109
P. serrana	Spain	MAF 9758	AY295108
P. submontana	Poland	UGDA L-21237	KU845682
P. submontana	Spain	MAF 10233	AY579457
P. sulcata	Denmark	UGDA L-21204	KU845628

Results

The best-scoring tree of the ML analysis, with the bootstrap support values of the main branches are shown in Fig. 1. Our six sequences fell within three highly supported clades, two in each. The three clades contained the epi- or holotypes of *P. ernstiae*, *P. saxatilis* and *P. serrana*, respectively.

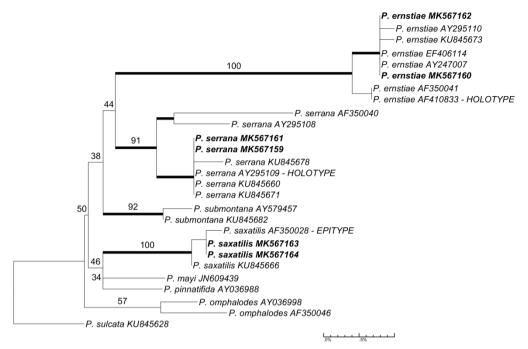


Figure 1. The best-scoring tree of the RAxML analysis of the nrITS sequences, with bootstrap support values on the main branches. Branches with support values ≥ 70 and newly generated sequences are in bold. The branch length of the outgroup (P. sulcata) is shortened.

The chemical analysis of the six sequenced specimens showed fatty acids in the four in the *P. ernstiae*- and *P. serrana*-clades, and no fatty acids in the two in the *P. saxatilis*-clade. Further analyses of 57 selected specimens of *P. saxatilis* s. lat. in O revealed 29 additional specimens containing fatty acids (mainly protolichesterinic and lichesterinic acid, the former in highest concentration).

The result of the morphological analysis is shown in Table 2, Fig. 2 (full character set), and Fig. 3 (reduced, 5 character set). In the full character set, the sum of scores varied from -3 to +4: 11 were negative (indicative of *P. serrana*), 12 were positive (indicative of *P. ernstiae*), 7 were zero (indecisive), and the four specimens identified by DNA sequence data were given scores of +2 (*P. ernstiae*, O L-201279, and *P. serrana*, O L-205007) and zero (*P. ernstiae*, O L-201277, and *P. serrana*, O L-195995).

Discussion

Our six sequenced specimens belong in three highly supported clades together with the epi- or holotypes of *P. ernstiae*, *P. saxatilis*, and *P. serrana*, respectively. The clades fit well the species concepts of Thell et al. (2017) and Ossowska et al. (2018), and *P. ernstiae* and *P. serrana* are hence here reported as new to Norway from two collections each.

Assuming the presence of fatty acids is diagnostic for *P. ernstiae* and *P. serrana* versus *P. saxatilis* (Ossowska et al. 2018), we also report 29 additional specimens of those two from Norway.

Table 2. Morphological and chemical characters scored in 30 specimens of *Parmelia ernstiae* and *P. serrana*. A score of 1 indicates the former species, –1 the latter, and 0 is indecisive in the species circumscriptions of Ossowska et al. (2018) and partly Thell et al. (2017). Four specimens are additionally identified by DNA data.

Specimen	Lobaric acid	Largest lobe	Lobules press.	Isidia margina,	Sual on lobes Isidia maints	Isidia on rides	Pruina presen.	Sum of scores	, Detei	mination
	Yes: 0 No: -1	≤3: 1 >3: -1	Yes: 1 No: -1	Yes: -1 No: 1	Yes: 1 No: -1	Yes: -1 No: 1	Yes: 1 No: -1		Score	DNA
O L-24672	0	-1	1	-1	1	1	-1	0	indet.	
O L-26146	-1	1	1	-1	1	1	-1	1	P. ernstiae	
O L-26564	0	1	1	-1	1	-1	1	2	P. ernstiae	
O L-26593	0	1	1	-1	1	-1	-1	0	indet.	
O L-33977	-1	-1	1	-1	-1	1	-1	-3	P. serrana	
O L-73208	-1	1	1	-1	-1	-1	-1	-3	P. serrana	
O L-73209	0	1	1	-1	1	1	-1	2	P. ernstiae	
O L-73383	0	1	1	-1	1	1	-1	2	P. ernstiae	
O L-73900	-1	1	1	-1	-1	1	-1	-1	P. serrana	
O L-74776	-1	1	1	-1	-1	-1	-1	-3	P. serrana	
O L-75634	0	1	1	-1	-1	1	-1	0	indet.	
O L-102565	-1	-1	1	-1	-1	1	-1	-3	P. serrana	
O L-108956	0	1	-1	-1	-1	1	-1	-2	P. serrana	
O L-121443	-1	-1	-1	-1	1	1	-1	-3	P. serrana	
O L-147773	0	1	1	-1	1	1	1	4	P. ernstiae	
O L-147789	-1	1	1	-1	1	1	-1	1	P. ernstiae	
O L-147941	-1	1	1	-1	-1	1	-1	-1	P. serrana	
O L-150681	0	1	1	-1	-1	1	-1	0	indet.	
O L-154722	-1	1	1	1	-1	1	-1	1	P. ernstiae	
O L-176945	0	1	1	-1	-1	1	1	2	P. ernstiae	
O L-177082	0	1	-1	-1	-1	1	-1	-2	P. serrana	
O L-177088	0	1	1	-1	-1	1	1	2	P. ernstiae	
O L-195995	0	-1	1	1	1	-1	-1	0	indet.	P. serrana
O L-198460	0	1	1	-1	-1	1	-1	0	indet.	
O L-201277	0	1	1	-1	-1	1	-1	0	indet.	P. ernstiae
O L-201279	0	1	1	-1	-1	1	1	2	P. ernstiae	P. ernstiae
O L-204021	0	1	1	-1	1	1	1	4	P. ernstiae	
O L-205007	0	1	1	1	1	-1	-1	2	P. ernstiae	P. serrana
O L-210114	0	-1	-1	-1	-1	1	1	-2	P. serrana	
O L-222474	0	1	1	-1	-1	-1	-1	-2	P. serrana	

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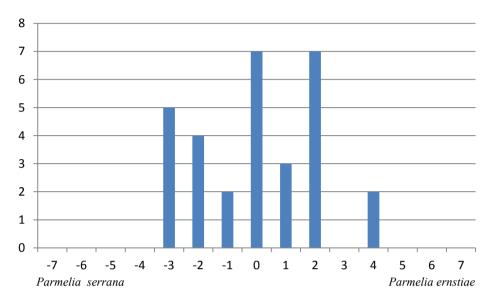


Figure 2. Number of specimens at each sum of scores for the seven morphological and chemical characters of the 30 specimens of *P. ernstiae/serrana* in Table 2. A positiv sum indicates *P. ernstiae*, a negativ *P. serrana*.

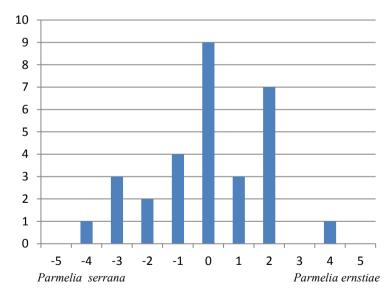


Figure 3. Number of specimens at each sum of scores for the five first morphological and chemical characters (i.e., those of Ossowska et al. 2018) in the 30 specimens of *P. ernstiae/serrana* in Table 2. A positiv sum indicates *P. ernstiae*, a negativ *P. serrana*.

Separating *P. ernstiae* and *P. serrana* on morphological characters, however, turned out to be difficult. In the full character set, there was no aggregation of the sum of scores at each end of the axis (Fig. 2); actually there was no sum of scores in the value intervals +5 to +7 and -4 to -7, and there were seven specimens with the sum of scores at value zero (fully indecisive). Our four DNA-identified specimens ended up on the axis as one correct, one erroneous, and two indecisive.

Removing the two characters which were not included by Ossowska et al. (2018) among the diagnostic characters (isidia on ridges and presence of pruina) did not make identification easier, as the sum of scores were still aggregated in the centre of the axis (Fig. 3). Actually, the number of specimens at value zero rose to 9, our two specimens of DNA-identified *P. serrana* ended up on the wrong side of the axis at values +2 and +4, respectively, and the two *P. ernstiae* at value zero. Hence, we refrain from identifying the 29 specimens containing fatty acids to the species level as the morphological analysis must be regarded as failed.

It appears to be very difficult to distinguish *P. ernstiae* and *P. serrana* on morphological characters. Actually, we were also unable to predict the absence of fatty acids in the specimens of *P. saxatilis* s. lat. prior to the TLC analysis, i.e., to recognize *P. saxatilis* s. str. on morphology alone. Perhaps a better trained eye can recognize the subtle morphological differences reported to exist between the three species, but we would advocate great caution in identifying material without DNA analysis, and regard them, for most practical purposes, as two, perhaps three, morphologically cryptic species. Still, we acknowledge that subtle morphologically differences may exist that are indicative in in mixed populations.

Material from most Norwegian counties was investigated by TLC but all the specimens belonging to the *P. ernstiae/serrana* complex were from low altitude and coastal localities. This indicates that these species mainly have southern and coastal distribution in Norway. We suspect the two species are common on bark of various deciduous trees in these regions and often occur intermixed with *P. saxatilis* which also grows in the same habitats.

Specimens examined: Parmelia ernstiae: **Norway**, *Nord-Trøndelag*: Flatanger, NE of lake Floavatnet, 64.4440°N, 10.7866°E, 50 m alt., 2015-08-05, R. Haugan et al. WG3-625 (O L-201277) [OLICH3848]; same locality, 64.4438°N, 10.7865°E, 30 m alt., 2015-08-05, M. Bendiksby et al. WG2-2074 (O L-201279) [OLICH2777].

Parmelia saxatilis: Norway, Buskerud: Sigdal, Sandvassetra, 60.3158°N, 9.3022°E, 1000 m alt., on stone on the ground in low alpine heath, 2013-09-29, S. Rui & E. Timdal 13184 (O L-184724) [OLICH940]; Sør-Trøndelag: Ørland, Storfosna, Haugan, 63.6683°N, 9.4045°E, 15 m alt, on roof of an old building, 2014-08-06, R. Haugan WG3-0100 (O L-195994) [OLICH1839].

Parmelia serrana: Norway, Vestfold: Larvik, Bøkeskogen, 59.0586°N, 10.0250°E, 60 m alt., on trunk of Fagus in beach forest, 22-Aug-2016, S. Rui & E. Timdal 16224 (O L-205007) [OLICH4004]; Sør-Trøndelag: Ørland, Storfosna, Haugan, 63.6683°N, 9.4045°E, 15 m, on roof of an old building, 2014-08-06, R. Haugan WG3-0101 (O L-195995) [OLICH1840].

Parmelia ernstiae/serrana: Norway, Aust-Agder; Lillesand, Vestre Moland church, 58.2569°N, 8.3609°E, 40 m alt., corticolous on Acer platanoides, 1996-09-13, H. Bratli 973 (O L-026564); Lillesand, near Justøy church, 58.2053°N, 8.3628°E, 20 m alt., corticolous on Alnus glutinosa, 1996-09-14, H. Bratli 1002 (O L-026593); Hordaland: Kvinnherad, Svartatjørna, 60.0519°N, 5.8556°E, 205 m alt., på hassel ved bekk, bjørk-ask-hasselskog, 2016-06-08, Jon T. Klepsland JK16-325 (O L-222474); Nord-Trøndelag: Leksvik, Stranda kirke, Vanvikan, 63.5537°N, 10.2203°E, 30 m alt., på platanlønn (Acer pseudoplatanus), kirkegård, 2006-07-18, B.P. Løfall bpl-L11143 (O L-147789); Røyrvik, Namsvatnet at the outlet of Vierma, MGRS: VN 4314-4315, alt. ca. 570 m, on Picea abies, 1974-07-11, T. Tønsberg 259 (O L-75310); Nordland: Dønna, Dønna gård, 66.2038°N, 12.5844°E, 25 m alt., på svenskasal (Sorbus intermedia), eldre parkanlegg, 2006-07-25, B.P. Løfall bpl-L11295 (O L-147941); Oslo: Bekkelaget kirke, 59.8537°N, 10.8019°E, 140 m alt., bjørk, 1972-09-29, V.I. Hansen s.n. (O L-121443); Rogaland: Bjerkreim, Odlandstø, 58.5935°N,

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6.1352°E, on *Ouercus*, 1971-04-06, H. Østhagen 211 (O L-073383); Forsand, Rossavika, ovenfor gårdene, LL 3523 2928, 85 m alt., på eik i hagemark, 2012-06-25, T. Høitomt L286Høi (O L-210114); Gjesdal, Oltesvika, 58.8506°N, 6.1268°E, 70 m alt., på rogn, beitemark med spredte trær, 2013-10-09, J.T. Klepsland JK13-L716 (O L-198460): Lund. Eikeland v/Malåna. Moi (Lundeskogen). 58.4484°N. 6.5471°E. grov syartor v/bekk, 2001-08-06, A. Lie s.n. (O L-108956); Suldal, Barkeland nature reserve, 59.3415°N, 6.1532°E, 85 m alt., on Ouercus sp., 1997-08-13, H. Bratli 4028 (O L-102565); Suldal, W of Ersdalsstølen, c. 7 km SSW of Sand, 59.4240°N, 6.2256°E, 250 m alt., on *Ulmus glabra*, 1996-08-02, R. Haugan 5123 (O L-026146); Sogn og Fjordane: Flora, Florø, ved buss-stasjonen, 61.6000°N, 5.3667°E, på Ulmus glabra, stamme, 1973-07-04, P. Størmer s.n. (O L-073900); Flora, Standalsvatnet V, 61.5123°N, 5.2332°E, 6 m alt., på eldre svartor (Alnus glutinosa), smal stripe med gråor-heggeskog, svartorutforming, langs middelstor bekk i kulturlandskapet, 2007-06-05, J.T. Klepsland JK07-L017 (O L-154722); Hornindal, Hornindal kirke, 61.9762°N, 6.6355°E, 55 m alt., på svenskasal (Sorbus intermedia), kirkegård, 2006-07-16, B.P. Løfall bpl-L11127 (O L-147773); Sør-Trøndelag: Trondheim, Leinstranden: Leinøra, 63.3419°N, 10.2505°E, 3 m alt., på Hippophaë rhamnoides, 1939-06-08, N. Skaanes s.n. (O L-074776); Ørland, Austråtskogen, 63.7064°N, 9.71827°E, 10 m alt., on trunk of old Tilia, 1995-10-02, R. Haugan, S. Rui & E. Timdal 8509 (O L-024672); Telemark; Seljord, Hønsegjuvet, 59.4552°N, 8.8580°E, 450 m alt., lindestamme i rasmarksskog med dominans av lønn og osp., 1999-04-25, R. Midteng 199 (O L-40611); Skien, Blåfjellstjern, 59.3874°N, 9.6099°E, kvist på levende bjørk, 1998-06-29, A. Heggland LF98X03-01/01 (O L-033977); Vest-Agder: Farsund, Lista, Lunde, S of Vollmoen, 58.0775°N, 6.7797°E, 5 m alt., on Acer pseudoplatanus in open situation, 1993-05-29, R. Haugan & O. Pedersen 2984 (O L-075634); Farsund, Sandvik, 58.1128°N, 6.8191°E, 40 m alt., på *Quercus* sp., 1977-05, O. Jølle s.n. (O L-073209); Farsund, Spind, Reisvåg, 58.0870°N, 6.8885°E, 30 m alt., 1993-05-31, R. Haugan 7501 (O L-150681); Farsund, Øyna, 58.1122°N, 6.7851°E, 52 m alt., på stamme av Quercus petraea, 1977-05, O. Jølle s.n. (O L-073208); Flekkefjord, Ysthus Ø (Hidra), 58.2269°N, 6.5363°E, 70 m alt., på rogn, skogkant/kant av beitemark, 2011-07-05, J.T. Klepsland JK11-L240 (O L-176945); Kristiansand, Grovannet SØ, 58.1936°N, 8.0103°E, 100 m alt., på eik, eldre blåbær-smyle-eik-furuskog, 2011-09-27, J.T. Klepsland JK11-L462 (O L-177082); Lindesnes, Tjøm, 58.0343°N, 7.2810°E, 15 m alt., på eldre rogn, utkant av storvokst, eldre blåbær-smyle-furuskog med gran, 2011-09-27, J.T. Klepsland JK11-L468 (O L-177088); Østfold: Aremark, Lundsneset naturreservat, SV-skrenten av Duvleøfse, 59.0411°N, 11.7146°E, 190 m alt., over mose på eik, 2000-06-15, H. Bratli 4623 (O L-65904); Halden, Hauglund, 59.0557°N, 11.6612°E, 220 m alt., trunk of Sorbus aucuparia, solitary tree in a meadow, 2015-02-26, R. Haugan 150172 (O L-204021).

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