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DNA barcoding confirms the first record of a *Desmodema polystictum* (Ogilby, 1898) egg and all-time high adult catches in the Indian Ocean

M. I. G. Rathnasuriya[1,2*], A. Mateos-Rivera[3*], A. G. G. C. Bandara[1], R. Skern-Mauritzen[3], R. P. P. K. Jayasinghe[2], J. O. Krakstad[3] and P. Dalpadado[3]

Abstract

The eggs of Polka-dot ribbonfish *Desmodema polystictum* have been recorded for the first time in the Indian Ocean. Although the only previous information on eggs from this species consists of line drawings from 1973 (Pacific Ocean), the identification was possible by combining morphological and molecular analyses. As far as we are aware, only few confirmed records of adult individuals have been previously reported from the Indian Ocean. We found eggs in the proximity of numerous adults (57 and 42 individuals at two stations) indicating that the Central Indian Ocean is potentially an important spawning ground of *D. polystictum*.

Keywords: Central Indian Ocean, COI gene, Fish egg, Mesopelagic fish, Trachipteridae

Background

The ribbonfish family Trachipteridae (Lampriformes) consists of three genera *Trachipterus* (*T. altivelis*, *T. arcticus*, *T. fukuzakii*, *T. ishikawae*, *T. jacksonensis*, *T. trachypterus*), *Desmodema* (*D. lorum*, *D. polystictum*), and *Zu* (*Z. cristatus*, *Z. elongatus*) (Martin 2015; Froese and Pauly 2019). Like most other species in this family, Polka-dot ribbonfish *Desmodema polystictum* (Ogilby, 1898) is distributed circumglobally in tropical and temperate regions (Martin 2015; Angulo and López-Sánchez 2017). However, despite its wide distribution and size, with lengths up to 110 cm, *D. polystictum* is an uncommon, deep dwelling species (Smith-Vaniz 2015). Although the first record of a single specimen of this species (a juvenile) was reported at the end of the twentieth century from the North Indian Ocean (Bauchot and Bianchi 1984), the presence of adults at other locations within the Indian waters were only published very recently (Zacharia and Kannan 2012; Deshmukh et al. 2017). There are few other records only available in reports and online databases. Two records of a single specimen of *D. polystictum* were from the waters of Pakistan in 2010 and 2015 (Fanning et al. 2011; Froese and Pauly 2019). Furthermore, eight individuals of this species have been found off Tanzanian waters (6° 43′ S, 43° 59′ W) in a survey conducted by the R/V Dr. Fridtjof Nansen (Institute of Marine Research (IMR), Norway-database, survey no: 2018405).

The Indian Ocean (Arabian Sea), is viewed as one of the most productive oceans regarding mesopelagic fish (FAO 2001). Until recently, there was comparatively little research activity on the mesopelagic zone worldwide (Hildago and Browman 2019). In the present study, both morphological and molecular approaches, targeting the mitochondrial cytochrome c oxidase 1 (COI) gene (Ward et al. 2009), were used in parallel for identifying a single egg of *D. polystictum* from samples collected in the Indian Ocean. Another egg was identified only based on its morphological characteristics. Furthermore, to identify the possible spawning areas, we explored the spatial overlap of adult fish, based on fieldwork and

* Correspondence: ishara.ruh@gmail.com; alejandro.mateos.rivera@hi.no
[1]Faculty of Fisheries and Ocean Sciences, Ocean University of Sri Lanka, Mahawela Road, Tangalle 82200, Sri Lanka
[2]National Aquatic Resources Research and Development Agency (NARA), Crow Island, Colombo 15, Sri Lanka
[3]Institute of Marine Research (IMR), P O Box 1870 Nordnes, N-5817 Bergen, Norway

literature review, in relation to the records of eggs from this survey.

Material and methods

Ichthyoplankton samples were taken with the R/V Dr. Fridtjof Nansen ecosystem survey around Sri Lankan waters of the Indian Ocean from 24 June to 16 July 2018 (Krakstad et al. 2018). A Continuous Underway Fish Egg Sampler (CUFES) was used to collect eggs and larval fish in the upper 4 m at 54 stations. Samples were preserved in 96% ethanol onboard and later processed at the laboratory of the National Aquatic Resources Research and Development Agency (NARA), Sri Lanka. The 3735 fish eggs found in the CUFES samples were divided into categories based on their morphological and meristic characteristics following Rodriguez et al. (2017). Among these eggs, only one, collected at 8° 48′ N and 79° 30′E, showed an unusual pattern of conspicuous branched melanophores and size of ca. 2 mm (Table 1a, b). In addition, a Multinet Mammoth (1 m^2 – Hydro-Bios) was towed obliquely from 25 to 0 m and 100–0 m respectively with bottom depth close to 30 m and > 100 m at 24 stations. At the station 576 (7° 40′ N, 79° 37′ E, Table 1a, b), a fish egg bearing very similar characteristics to the one collected by the CUFES was retrieved. It was not possible to obtain DNA sequence of this egg as it was subsequently lost after the survey.

Two pelagic trawls (Åkrahamn - mouth opening 8 to 12 m and MultPelt 624 mouth opening 25 to 35 m) and a Gisund Super bottom trawl (18.5 m horizontal opening, 4–5 m vertical opening) were used to catch fish during the same survey (Krakstad et al. 2018). In total, 88 trawl hauls were taken. To identify the *D. polystictum* adults, we used morphometric and meristic data (Table 2) following Zacharia and Kannan (2012). Temperature and salinity profiles were obtained by a Seabird 911 CTD at the same survey. Further information regarding the sampling strategy can be found in Krakstad et al. (2018). Additional data on the occurrence of this species in the Indian Ocean was obtained from previous studies (Bauchot and Bianchi 1984; Zacharia and Kannan 2012; Deshmukh et al. 2017, reports and online databases).

The egg taken with the CUFES samples was placed in a 1.5 ml Eppendorf tube containing 75 μl of a solution of 5% Chelex 100 Resin (BioRad, CA, USA) and 15 μl of Proteinase K (Qiagen, Germany). The Eppendorf tube was incubated at 56 °C for 1 hour followed by 10 minutes at 96 °C. After a brief centrifugation, the supernatant containing the nucleic acids was transferred into a new Eppendorf tube. DNA isolation and PCR application targeting the mitochondrial COI gene was preformed using methods described by Ivanova et al. (2007) and further modified at the IMR, Norway (Mateos-Rivera pers. com.). Finally, sequencing was performed using 1 μL of

Table 1 a – Sampling site information of the stations containing *Desmodema polystictum* eggs and adults from the R/V Dr. Fridtjof Nansen survey during 24 June-16.July 2018, **b** – Sampling information on *Desmodema polystictum* eggs and adults collected during the survey. Temperature and salinity were obtained at four different depths (5, 25, 50 and 100 m) from CTD stations at the proximity to the sampling location

a

Station	Date	Latitude N	Longitude E	Start time (GMT)	End time (GMT)	Bottom Depth (m)	Gear type
51	12/ 07/ 2018	8° 48′	79° 30′	12:10	15:15	291	CUFES
576	11/ 07/ 2018	7° 40′	79° 37′	07:06	07:08	38	Multinet Mammoth
65	7/ 07/ 2018	5° 54′	79° 57′	14:14	14:48	585	Pelagic trawl
80	10/ 07/ 2018	7° 29′	79° 25′	15:27	16:00	1772	Pelagic trawl
81	11/ 07/ 2018	8° 13′	79° 24′	15:36	16:22	1074	Pelagic trawl
87	13/ 07/ 2018	7° 35′	78° 58′	18:18	19:04	2304	Pelagic trawl

b

Station	Sampling depth (m)	Stage	No. of individuals	Average wt. individual (g)	Temperature (°C)				Salinity			
					5 m	25 m	50 m	100 m	5 m	25 m	50 m	100 m
51	4	Egg	1	–	27.49	27.5	27.45	19.42	34.98	34.98	34.98	34.94
576	30	Egg	1	–	27.31	27.31	22.8	19.61	34.96	34.96	34.82	34.87
65	30	Adult	2	580	27.07	26.68	23.15	20.93	34.61	34.64	34.76	34.84
80	10	Adult	57	313	26.49	26.49	24.32	19.83	35.08	35.08	34.9	34.95
81	10	Adult	1	580	25.85	25.8	22.01	18.83	34.97	34.97	34.85	34.98
87	10	Adult	42	502	26.22	26.2	22.41	18.54	35.07	35.07	34.91	34.91

Table 2 Morphometric and meristic measurements of preserved specimen of *Desmodema polystictum* (Ogilby, 1897) at the museum of the National Aquatic Resources Research and Development Agency (NARA), Sri Lanka, compared with the measurements of Zacharia and Kannan 2012

Characters	Measurements (cm)	Ratios	
Morphometric counts		Present specimen	Reference specimen
Total length (TL)	90.7		107
Head length (HL)	6.4	7.1% of TL	9.1% of TL
Body depth (BD)	7.8	8.6% of TL	10.7% of TL
Pre-pectoral length	6.8	7.5% of TL	6.9% of TL
Pre-anal length	29.4	32.4% of TL	
Length of pectoral fin	2.3	2.5% of TL	
Eye diameter	3.0	46.9% of HL	38.8% of HL
Pre-orbital length	2.5	39.1% of HL	30.6% of HL
Post-orbital length	2.7	42.8% of HL	
Meristic counts			
Pectoral fin rays	14		14
Dorsal fin rays	121		121
Gill rakers	12 (3 + 9)		12 (3 + 9)

M13F primer [0.35 μM] (Messing 1983) at the University of Bergen (http://www.seqlab.uib.no). The resulting sequence was analyzed in Geneious v8.0.5 (Kearse et al. 2012) and used as query for both the Barcode of Life Data System (BOLD; www.boldsystems.org.) and BLASTn (Altschul et al. 1990) in GenBank (www.ncbi.nlm.nih.gov/genbank.) databases to determine its identity. The sequence has been submitted to GenBank under accession number MN117725.

The phylogenetic tree was made on selected COI gene sequences retrieved in the nucleotide database from NCBI. The tree was reconstructed by using maximum likelihood analysis and the Jukes-Cantor nucleotide substitution model as implemented in MEGA v7 (Kumar et al. 2016). Robustness of the tree was determined using 1000 bootstrap replicates. Sequences were aligned with MUSCLE (Edgar 2004) in MEGA v7. The tree was rooted against the COI gene sequence of an euphausiid (*Thysanoessa raschii*).

Results and discussion

The two *D. polystictum* pelagic eggs collected in this study were found in the North-West waters of Sri Lanka in the Central Indian Ocean close to the continental shelf/slope (Table 1b, Fig. 1a). Generally, during the southwest monsoon period when the survey was undertaken, the waters to the east of Sri Lanka are much warmer than to the west (approximately 29 vs 27 °C) (Rathnasuriya et al. submitted). The water temperatures at the two stations (51 and 576) at the time of sampling of *D. polystictum* eggs, varied from around 27.3 °C at the surface to 19.4 °C at 100 m depth while the salinity varied from 34.96 to 34.87 respectively at surface and 100 m (Table 1b). The temperature and salinity conditions at the two sampling stations mentioned above reflects the overall situation observed along the west coast (Krakstad et al. 2018). During the survey, a cooler temperature signal was identified in the far offshore North West region, reflecting more upwelling and likely associated increase in biological productivity in the region (Krakstad et al. 2018; Rathnasuriya et al. submitted). The high biomass of pelagic and mesopelagic fish was observed in offshore waters is likely due to favorable feeding conditions in the area. Prior to this survey, there were no available data on ichthyoplankton from this region (Krakstad et al. 2018).

The egg of *D. polystictum* showed a unique pattern of conspicuous branched melanophore scattered over the yolk, anterior to eye and over the head and lateral sides of developing embryo (Fig. 2a). Lateral melanophores on the developing embryo were arranged in several rows from head to tail dorsally on epaxial myomeres, and ventrally on hypaxial myomeres. A transparent chorion was also observed, with narrow perivitelline space, and homogenous yolk with no oil globule present (Fig. 2a, b). This description of the egg agrees with the drawings in Pertseva-Ostroumova and Rass (1973), which to our knowledge is the only previous information about *D. polystictum* eggs. However, no specimens from that study are available. As the morphological identification of fish eggs for the first time can be tricky, we used DNA barcoding to verify our findings. In addition, we emphasize the need to use molecular analysis, such as DNA barcoding to successfully identify fish eggs as well as to incorporate them into long-term, regional scale ichthyoplankton monitoring programs as has been suggested in recent studies (Lewis et al. 2016; Burrows et al. 2019).

After sequencing and cleaning the COI gene of the egg, we obtained a sequence of 658 bp which was blasted against the GenBank and BOLD databases. Our query sequence showed a high identity (> 99%) with 3 sequences deposited in the GenBank database identified as *D. polystictum* (accession numbers: MG856425; MG856804 and KF489564; Fig. 3). In addition, the same high sequence identity was obtained with one sequence deposited in the BOLD database (id: MFLE4752) and associated only to the genus *Desmodema* from an individual collected in the Western part of Jamaica in 2011. Judging from our results, the sequence from the Caribbean would also belong to *D. polystictum*. However, to our knowledge, none of the above four sequences have been used in any published

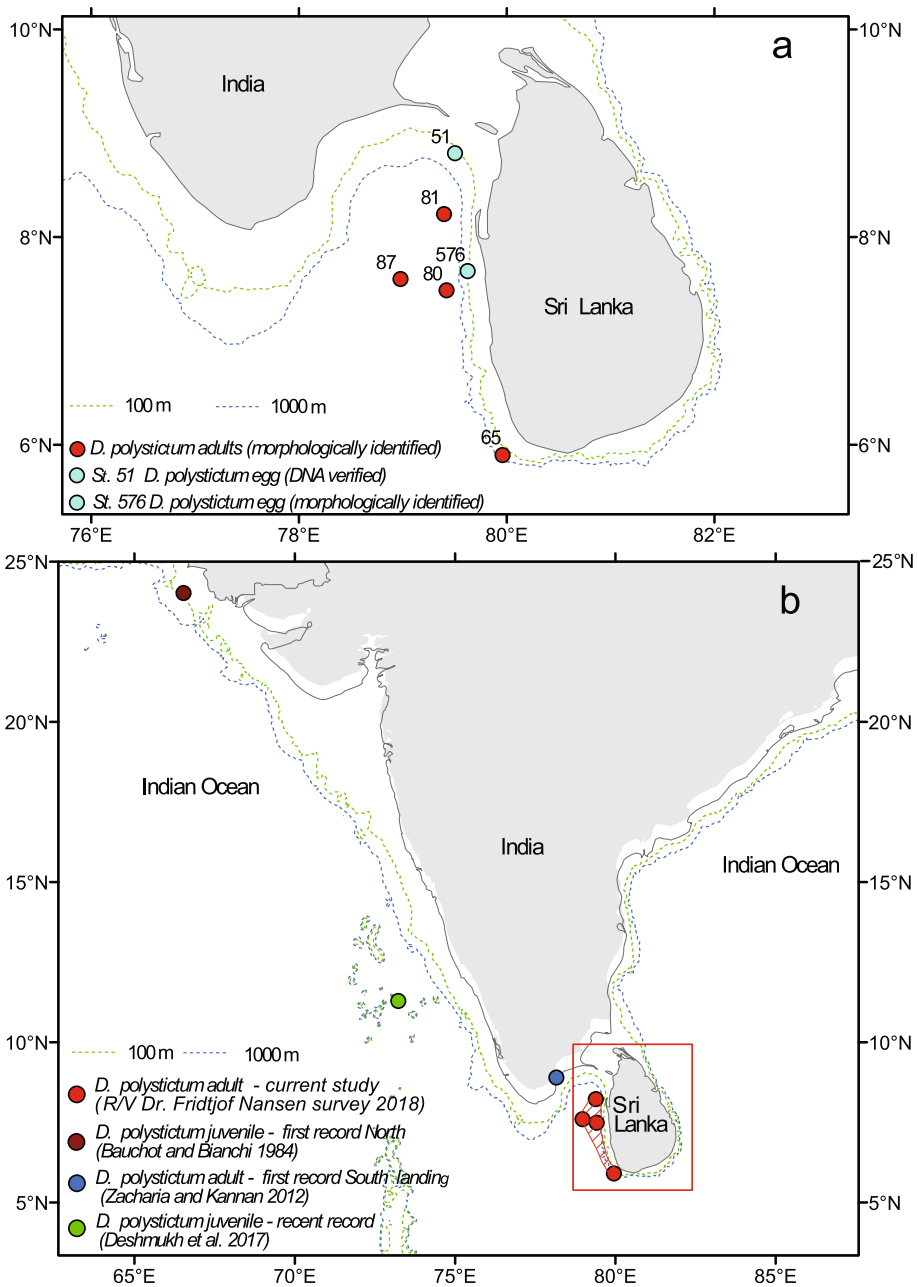

Fig. 1 Map with the location of the stations where *Desmodema polystictum* have been collected. **a** – eggs, caught with CUFES and Multinet Mammoth, (Turkish blue circle), and the adults, caught with pelagic trawl (red circle), **b** – adults recorded in the Indian Ocean from the current (red) and previous (brown, blue and green) studies. Records available in reports and databases were not incorporated

study so far. In addition, lower identities were obtained with sequences from other species such as *D. lorum* (accession number: GU440303) or *Zu cristatus* (accession numbers: KC016059, KU836510, KF930551), among others (Fig. 3).

Among the 88 trawl stations (pelagic and demersal) taken during the R/V Dr. Fridtjof Nansen survey, *D. polystictum* adults were only observed in offshore waters (> 600 m bottom depth) in 4 stations (Table 1a, b, Fig. 1a, b, Fig. 4). All individuals were caught by pelagic trawls covering the upper 30 m during nighttime (19:00–23:30 local time; data are available at the IMR, Norway-database). Therefore, *D. polystictum* appear to follow the pattern of other mesopelagic fish, which are known in general to perform diel vertical migration between the deeper waters (~ 200–1000 m), where they hide from predators during the day, and the oceanic surface layer where they feed at night (Aksnes et al. 2017).

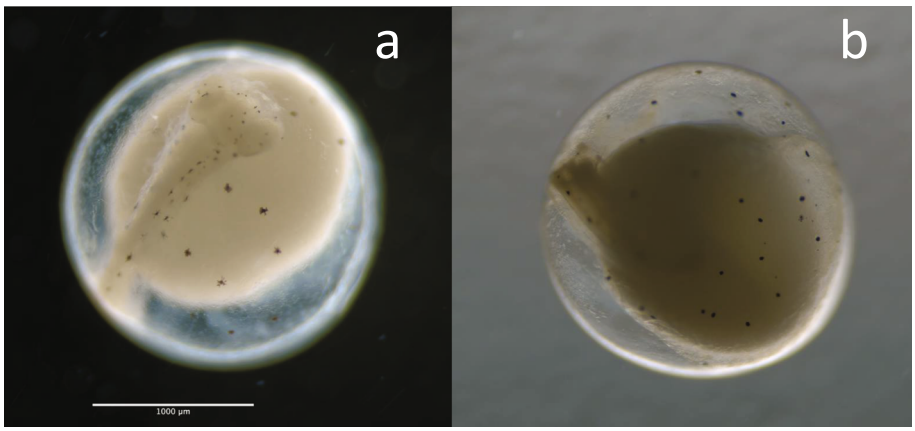

Fig. 2 *Desmodema polystictum* egg in late developing stage (tail but free from yolk), caught by CUFES showing the head and body of the embryo and the yolk. **a** – shows the pigmentation on the body of the embryo and the distinct branching melanophores over the head and lateral sides, **b** – shows the melanophores scattered over the yolk

The majority of the adults were collected at stations 80 and 87 (57 and 42 individuals), respectively; Table 1(b). While, in the other two stations, 65 and 81, the number was considerably lower (2 and 1, respectively). In fact, *D. polystictum* is a rare, deep dwelling species (Smith-Vaniz 2015), which can be confirmed by the little information available about this species. Indeed, to our knowledge, only few individuals have been previously reported in the Indian Ocean (Bauchot and Bianchi 1984; Zacharia and Kannan 2012; Deshmukh et al. 2017, reports and online databases).

The western region of the Indian Ocean close to Sri Lanka is a highly productive area (Yapa 2012), making this zone attractive as a feeding ground for fish. Good feeding conditions (e.g. high biomass of mesozooplankton) likely attracts early larval stages into the inner shelf regions (Krakstad et al. 2018). Though sparse, records of eggs close to the continental shelf /slope may imply that this pattern is also likely adapted by *D. polystictum.* Eggs of some species may occur both on the continental shelf and over deep ocean waters while other species may restrict their spawning to the continental break (Burrows et al. 2019). The eggs recorded near the shelf break and shelf in our study seem to indicate that these could be dispersed from offshore waters (where adults were abundant) with the near surface northward flowing currents closer to the shelf (Krakstad et al. 2018). It is likely that spawning of *D. polystictum* coincided with the southwest

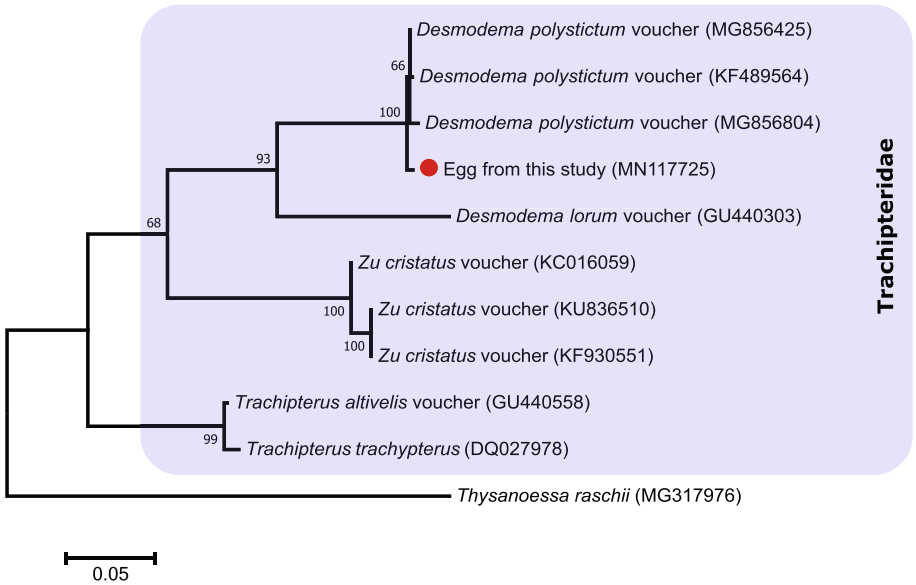

Fig. 3 Maximum-Likelihood phylogenetic tree. Accession numbers are provided within parenthesis. The studied egg is marked with a red dot

Fig. 4 Adult *Desmodema polystictum* collected at the station 80 with the R/V Dr. Fridtjof Nansen survey, 24 June–16.July 2018. (courtsey of S.S. Gunasekara, NARA)

monsoon where high plankton production occurred (Rathnasuriya et al. submitted) in the southern and western regions off Sri Lanka. The high density of adults found in two of the sampling sites could indicate seasonality or a coordinated reproductive event, given the suitable environmental conditions. This could be a strategy of adults to provide good feeding conditions and high survival rates for larval fish. It is noteworthy that all adults of *D. polystictum* reported to date are from the western Indian Ocean, west side of India and Sri Lanka (Fig. 1b). The geographical overlap of eggs and adults in the Indian Ocean (North West region off Sri Lanka) indicates that this area could be an important spawning ground of *D. polystictum.*

Conclusion

This study contains novel findings of a rare mesopelagic species that is distributed circumglobally in tropical and temperate regions. Our findings verify for the first time, the eggs of *D. polystictum* through morphological and DNA barcoding approaches. We observed significantly higher number of adults than previously reported from the Indian Ocean. The high density of adults found in the western region may indicate spawning aggregations in an area with favourable environmental conditions for their offspring. Eggs in the proximity of numerous adults indicate that the Central Indian Ocean is likely an important spawning ground of *D. polystictum*.

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Authors' contributions

In situ data collection and database management: MIGR; RPPKJ; JOK; PD. DNA barcoding: AMR; RSM. Formal analysis: MIGR; AMR; AGGCB; PD. Writing – review & editing: MIGR; AMR; AGGCB; RSM, RPPKJ; JOK; PD. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval

All animals we have used in this study are collected from sea, in their natural environment in accordance with the national and international regulations.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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