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First report and characterization of adult *Uncinaria* spp. in New Zealand Sea Lion (*Phocarctos hookeri*) pups from the Auckland Islands, New Zealand

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Abstract Two species of hookworms (*Uncinaria lucasi* and *Uncinaria hamiltoni*) have been formally described from pinnipeds, but dissimilar types are noted from these hosts. This report is the first description of hookworms (*Uncinaria* spp.) from the New Zealand sea lion, *Phocarctos hookeri*. The nematodes were collected from dead pups on Enderby Island (Auckland Islands, 50°30', 166°17') during January and February, 2004. Standard measurements of male and female hookworms were obtained, providing a general morphometric characterization of the hookworm species in *P. hookeri*. Considerable variations in the body length of adult hookworms were noted within the same host. The arrangement of some of the bursal rays differs from that described for *U. lucasi* and *U. hamiltoni*.

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Introduction

The New Zealand Sea Lion (NZSL), *Phocarctos hookeri* (Hooker's sea lion), is one of the rarest and most locally endemic members of the Otariidae. This species has been classified as “vulnerable” and “endangered” because of its small population size and limited distribution on the sub-antarctic Auckland Islands (Gales and Fletcher 1999). Studies conducted during the past 7 years on Enderby Island (Auckland Islands) have shown that hookworm infection is a significant cause of mortality in pups (Castinel et al. 2004).

Only two hookworm species, *Uncinaria lucasi* Stiles and *Uncinaria hamiltoni* Baylis, have been described from otariids (Stiles 1901; Baylis 1933, 1947). However, individual hookworms with intermediate morphotypes have been reported from pinniped hosts (Baylis 1947; Olsen 1952; Dailey and Hill 1970; Nadler et al. 2000), suggesting that additional biodiversity may be present but, as yet, uncharacterized. The present study provides a morphometric description of hookworm specimens collected from infected NZSL pups on Enderby Island. This description is necessary for more comprehensive comparisons of *Uncinaria* spp from various pinniped hosts.

Materials and methods

Necropsies to investigate neonatal mortality were conducted in the field on NZSL pups found dead on Sandy Bay beach, Enderby Island (Auckland Islands, 50°30'; 166°17') during the period from January 7 to February 14 2004. Mature adult hookworms were collected from the intestinal tracts of 4- to 7-week-old pups and stored in 100% ethanol. Thirty mature specimens were selected from four different pups—in which individual burden ranged from 2,310 to 7,080 parasites—for morphological study, cleared with a few drops of lactophenol and mounted temporarily on slides. The hookworms were examined using a light microscope, and images were acquired using a digital camera. Morphological study concentrated on features used pre-

viously to characterize *U. lucasi* and *U. hamiltoni*, including the buccal capsule, esophagus, male copulatory bursa, and female tail. Fertile eggs (eight-cell stage) were obtained and measured by dissection of the uteri of females. Esophageal length was measured from the beginning of the esophageal tube (at the base of the buccal capsule) to the junction with the intestine; this differs from the variable measured by Baylis (1933) and Olsen (1952). In addition, some specimens of both genders were prepared for scanning electron microscopy (SEM) following standard procedures. Eight adult males and 11 adult females were measured using a microscope equipped with an ocular micrometer (Table 1). Measurements (in text) are means followed by standard errors (ranges are provided in Table 1). Ratios of some features were used to compare measurements between males and females. Analyses consisted of descriptive statistics and one-way analyses of variance (ANOVAs) to investigate the effect of gender on the measured variables. Adult *Uncinaria* spp specimens of each sex were deposited as vouchers in the US National

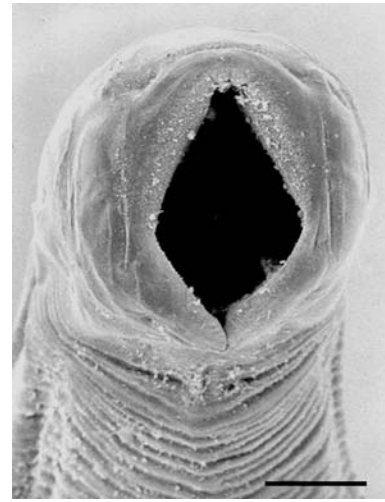


Fig. 1 SEM of the anterior end of an adult hookworm (female) from a NZSL pup in en face view. Bar scale: 40 μ m

Table 1 Measurements^a of *Uncinaria* spp. from NZSL pups, *U. hamiltoni* from *Otaria byronia* (Baylis 1933), *U. lucasi* from *C. ursinus* (Baylis 1947) and *U. hamiltoni* from *Otaria flavescens* (Berón-Vera et al. 2004)

	<i>Uncinaria</i> spp. from <i>Phocarcetos hookeri</i> (present study)		<i>Uncinaria</i> <i>hamiltoni</i> from <i>Otaria byronia</i> (Baylis 1933)		<i>Uncinaria lucasi</i> from <i>Callorhinus</i> <i>ursinus</i> (Baylis 1947)		<i>Uncinaria hamiltoni</i> from <i>Otaria</i> <i>flavescens</i> (Berón-Vera et al. 2004)	
	Females	Males	Females	Males	Females	Males	Females	Males
Sample size	11	8	3	?	?	?	30	29
Body length	10.35±56 7.20–12.30	6.82±0.49 5.00–9.20	12.5–17.5	8.5–12.0	12.4–16.0	7.4–8.7	11.37±2.94 5.36–17.2	7.85±1.52 4.88–10.64
Buccal capsule length	0.23±0.04 0.22–0.25	0.20±0.004 0.19–0.21	0.32–0.38	0.28–0.30	0.24–0.28	0.21–0.24	0.28±0.03 0.21–0.34	0.24±0.02 0.21–0.28
Buccal capsule width	0.19±0.007 0.16–0.23	0.17±0.005 0.15–0.19	–	–	–	–	0.24±0.02 0.20–0.31	0.19±0.06 0.14–0.24
Teeth height	0.044±0.007 0.037–0.050	0.030±0.002 0.025–0.035	–	–	–	–	–	–
Esophagus length	1.07±0.021 0.99–1.16	0.91±0.021 0.86–0.99	1.60–1.90	1.50–1.55	1.25–1.40	1.10–1.20	1.15±0.11 0.82–1.34	1.09±0.19 0.79–1.83
Diameter of esophageal bulb	0.18±0.003 0.17–0.20	0.16±0.004 0.14–0.18	–	–	–	–	–	–
Nerve ring from anterior end of body	0.69±0.020 0.56–0.78	0.52±0.002 0.37–0.74	0.60–1.10	–	0.57–0.62	–	–	–
Spicule length	–	0.69±0.016 0.62–0.74	–	1.000	–	500–560	–	0.89±0.09 0.57–1.05
Accessory piece length	–	0.060–0.080	–	0.12–0.14	–	80–90	–	–
Vulva to posterior end	4.01±0.16 3.19–4.84	–	5.10–7.00	–	5.20–6.30	–	4.33±1.34 2.13–6.62	–
Length of tail	0.20±0.007 0.16–0.23	–	0.16–0.25	–	0.21–0.25	–	–	–
Egg length	0.13–0.14	–	0.135–0.138	–	0.12–0.14	–	0.10–0.14	–
Egg width	0.072–0.081	–	0.085–0.093	–	0.080–0.088	–	0.04–0.10	–

^aMeasurements are given in millimeters; they are all ranges with additional means/standard deviations for NZSL hookworms

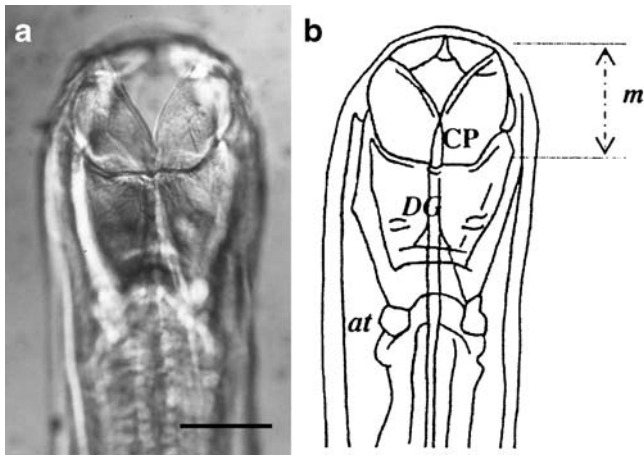


Fig. 2 Microphotograph (a) and drawing (b) of the buccal capsule of an adult hookworm (male) from a NZSL pup. Superficial dorso-ventral view showing the mouth opening (*m*), cutting plates (*CP*), dorsal gutter of esophagus (*DG*), and annular thickening (*at*) at the base of buccal capsule. Bar scale: 80 μ m

Parasite Collection (USNPC No. 96483) (Agricultural Research Service, US Department of Agriculture, Beltsville, MD, USA).

Results

All hookworm specimens presented the general characteristics of *Uncinaria* spp. (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9), with the typical anterior bend resulting in a dorsal buccal aperture (Fig. 1). The mouth opening spans about one half of the buccal capsule depth in dorso-ventral view. There are two pairs of cutting plates, one anterior and one posterior (Figs. 2b and 4b), the latter almost entirely visible through the mouth opening in dorso-ventral view (Lyons and

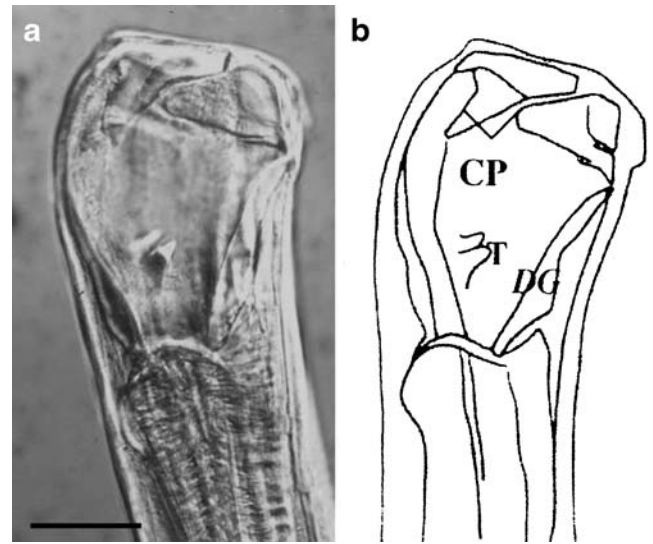


Fig. 4 Microphotograph (a) and drawing (b) of the buccal capsule of an adult hookworm (male) from a NZSL pup. Lateral view with the pair of teeth (*T*), cutting plates (*CP*), and dorsal gutter (*DG*). Bar scale: 80 μ m

Delong 2005). *Buccal capsule*: The buccal capsule is elongated; $202.0 \pm 2.0 \mu$ m long by $167.5 \pm 5.3 \mu$ m wide in males and $232.5 \pm 3.7 \mu$ m by $195.4 \pm 6.6 \mu$ m in females. There is a pair of very well-developed sub-ventral teeth (Figs. 3b and 4b), with lengths from 25 to 35 μ m in males and 27 to 50 μ m in females. There is an annular thickening of the wall at the base of the buccal capsule (Fig. 2b), and the dorsal gutter of the esophageal gland is pronounced (Figs. 2b and 4b). Cephalic sensilla visible on the cuticle include two pairs of papillae and one pair of amphids surrounding the border of the mouth opening.

Esophagus: The esophagus is flask-shaped, common to the other species of *Uncinaria*. The length (measured

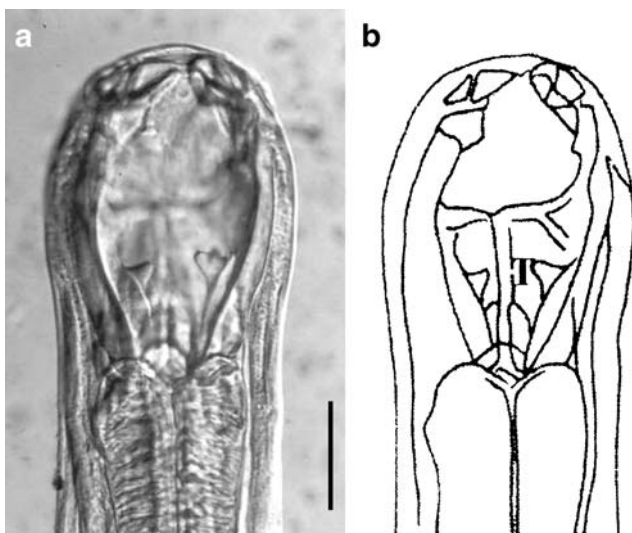


Fig. 3 Microphotograph (a) and drawing (b) of the buccal capsule of an adult hookworm (male) from a NZSL pup. Deeper dorso-ventral view underlining the pair of sub-ventral teeth (*T*). Bar scale: 80 μ m

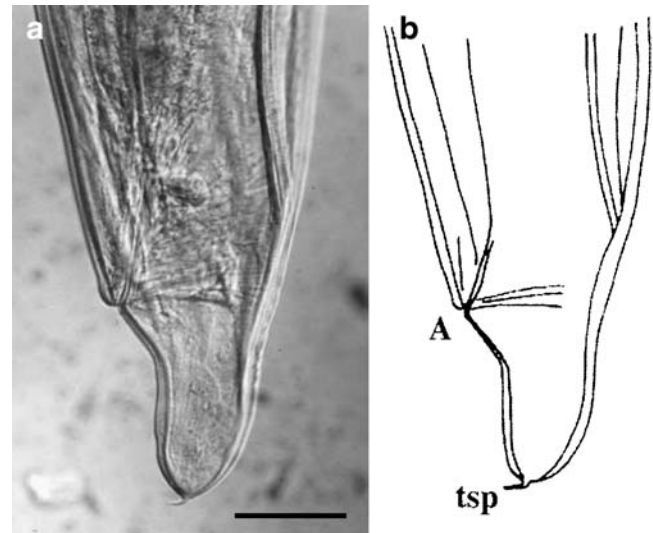


Fig. 5 Microphotograph (a) and drawing (b) of the terminal end of a female adult hookworm from a NZSL pup. Lateral view of the tail showing the anus (*A*) and the mucro or terminal spike (*tsp*). Bar scale: 80 μ m

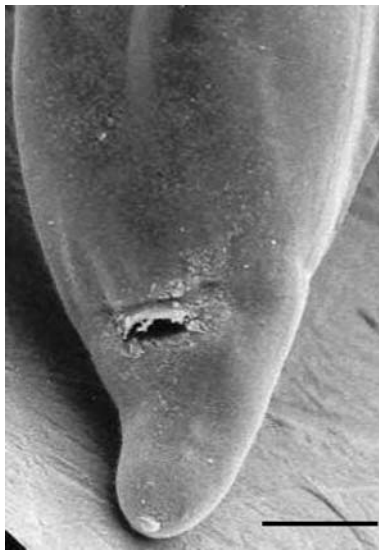


Fig. 6 SEM of ventral view of the tail of a female adult hookworm showing the anus. Bar scale: 50 μm

from anterior to posterior end) is $912.2 \pm 21.1 \mu\text{m}$ in males and $1,074.7 \pm 20.7 \mu\text{m}$ in females; the width at the base is $157.5 \pm 4.1 \mu\text{m}$ in males and $183.8 \pm 2.9 \mu\text{m}$ in females. The nerve ring surrounding the esophagus above the bulb region is at $526.2 \pm 70.4 \mu\text{m}$ from the anterior end of the body in males and at $685.5 \pm 22.5 \mu\text{m}$ in females.

Males: The males are $6.82 \pm 0.49 \text{ mm}$ long. The two copulatory spicules are equal in length ($688.5 \pm 16.3 \mu\text{m}$). The gubernaculum is 60 to 80 μm long, oblong, and widens posteriorly. The bursa (Fig. 7a) shares similar features with that of order Strongylida (Olsen 1974; Schmidt and Roberts 1989). The ventral rays are fused and measure from one half to two thirds of the medio-lateral ray (Fig. 8b). The ventral rays diverge perpendicularly from the antero-lateral-ray axis (Fig. 8b). The tips of the postero- and antero-lateral rays are directed away from the medio-lateral ray. The antero-lateral ray does not touch the edge of the lateral lobe. The dorsal ray bi-

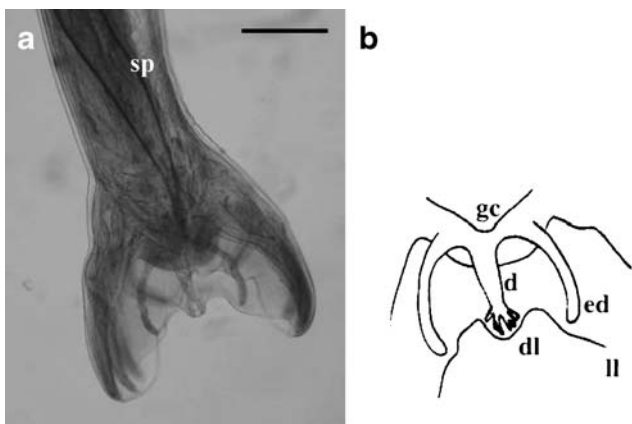


Fig. 7 Microphotograph (a) and drawing (b) of the terminal end of a male hookworm from a NZSL pup. Dorso-ventral view of bursa showing paired spicules (*sp*), genital cone (*gc*), dorsal (*dl*) and lateral (*ll*) lobes, and externo-dorsal (*ed*) and dorsal (*d*) rays. Bar scale: 150 μm

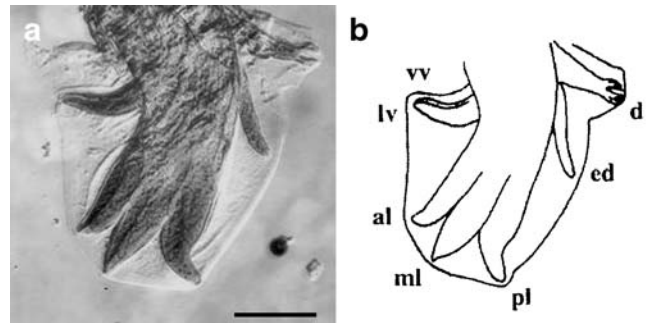


Fig. 8 Microphotograph (a) and drawing (b) of the terminal end of a male hookworm from a NZSL pup. Lateral view of copulatory bursa presenting the arrangement of ventro-ventral (*vv*), latero-ventral (*lv*), antero-lateral (*al*), medio-lateral (*ml*), postero-lateral (*pl*), externo-dorsal (*ed*), and dorsal (*d*) rays. Bar scale: 100 μm

furcates distally with three small branches at the tips, and diverges from the outer ray. The dorsal lobe is very small and semicircular. The externo-dorsal ray is long, reaching the margin of the lateral lobe, and diverges proximally from the dorsal ray. Paired caudal papillae are present on the lateral lobe.

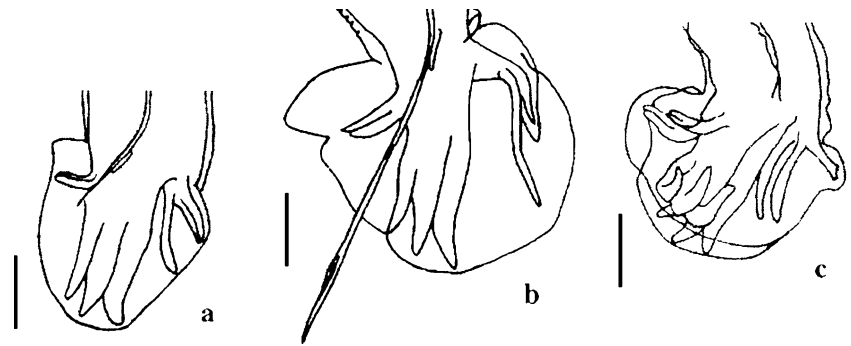
Females: The females are $10.35 \pm 0.56 \text{ mm}$ long. The vulva is prominent, and is located $4.01 \pm 0.16 \text{ mm}$ from posterior end. The vagina is short and wide. The ovejectors are longitudinal and symmetrical, with a combined length of 450 μm . The tail (anal opening to tail tip) is $196.2 \pm 6.8 \mu\text{m}$ long (Fig. 6). The tail mucro (Fig. 5b) is usually bent, and is between 10 and 30 μm long. A pair of caudal papillae is found at 38–40 μm from the terminal tip. The eggs are $136.6 \pm 0.7 \mu\text{m}$ long by $77.1 \pm 1.4 \mu\text{m}$ wide.

Results of statistical analyses are presented in Table 2 for both crude variables and ratios of the same parameters over the body length. There was a significant difference between males and females in body length ($p < 0.001$), buccal capsule length ($p < 0.001$) and width ($p < 0.001$), esophageal length ($p < 0.001$), the diameter of the esophageal bulb ($p < 0.001$), and the distance of the nerve ring from the anterior end ($p < 0.001$). Tests comparing the ratios of these variables to the total body length showed that gender had no effect on the position of the nerve ring ($p = 0.63$); although increasing towards the level of statistical significance, all other p values for these size characters still support differences between males and females. This suggests that ratios related to the body length could correct the bias caused by the variations of size in the specimens examined.

Discussion

The measurements of *Uncinaria* spp. from NZSL pups showed a strong sexual dimorphism (body length, buccal capsule length, and esophageal length), but there were some morphological features of the buccal capsule and the esophagus that did not differ between sexes. Likewise, gender had no effect on the position of the nerve ring when statistical comparison included the ratio of the distance as based on total body length. This indicates that the ratios of

Fig. 9 Lateral views of male copulatory bursae from *Uncinaria* spp (redrawn): **a** *U. hamiltoni* from Baylis (1933), **b** *U. lucasi* from Baylis (1947), **c** *U. hamiltoni platensis* from Botto and Mañe-Garzon (1975). Bar scale: 200 μ m



certain measured variables may provide more accurate conclusions than those conclusions based on specific measurements, especially when comparing *Uncinaria* spp. in pinniped hosts (George-Nascimento et al. 1992) where sexually mature hookworms can vary substantially in total body length. Ranges of body length in *Uncinaria* spp. from NZSL were similar to those from Berón-Vera et al. (2004), but were reduced relative to *U. lucasi* and *U. hamiltoni* as described by Baylis (1933, 1947). Almost all variables measured in hookworms from the southern hemisphere (Botto and Mañe-Garzon 1975; Berón-Vera et al. 2004) present a broad range with regard to measurements important for comparing species: buccal capsule length, esophagus length, distance of vulva to posterior end, spicule length, and egg dimensions (Table 1). Disparity in the size of mature hookworms within the same host was observed during the present study. The measurements from *Uncinaria* spp. found in the Northern Hemisphere show less variation (Nadler et al. 2000). Lyons (2005) quotes

Olsen (1952): “While the body size of the hookworms from the sea lions tends to be larger than those from the fur seals, the relative proportions on a percentage basis remain almost the same.” This pertains to *U. lucasi*, from northern fur seal (*Callorhinus ursinus*) and Steller sea lion (*Eumetopias jubatus*) pups from St. Paul Island, Pribilof Islands, Alaska (Olsen 1952). Using ratios may permit compensation for this type of bias. It seems inappropriate to include body width in comparisons for *Uncinaria* spp. in NZSL, not only because mounting specimens between a slide and a cover slip is highly likely to deform the diameter of a nematode, but because previous authors do not identify the precise site of body width measurement.

The arrangement of the bursal rays of *Uncinaria* spp in the NZSLs is characterized by the configuration of lateral rays resembling *U. lucasi*, and the externo-dorsal ray being much longer than the dorsal one is a feature more similar to *U. hamiltoni*. This reinforces the need for an extensive morphometric examination of *Uncinaria* species in pinnipeds.

Table 2 Effect of gender on measurements of *Uncinaria* spp. from NZSL pups

Variables	Difference between sexes (<i>p</i> value) for crude variables	Ratio to body length		Difference between sexes (<i>p</i> value) for ratio to body length
		Male	Female	
Body length	0.00015	–	–	–
Buccal capsule length	0.0000013	0.020–0.033	0.019–0.033	0.0212
Buccal capsule width	0.00657	0.017–0.032	0.017–0.022	0.000576
Esophagus length	0.000361	0.097–0.173	0.084–0.151	0.00417
Diameter of esophageal bulb	0.0000673	0.027–0.030	0.014–0.023	0.00920
Nerve ring to anterior end of body	0.015	0.060–0.088	0.053–0.096	0.6259
Spicule length	–	0.077–0.113	–	–
Vulva to posterior end of body	–	–	0.346–0.442	–
Tail	–	–	0.014–0.026	–

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