NASO-PHARYNGEAL MITES *HALARACHNE HALICHOERI* (ALLMAN, 1847) IN GREY SEALS STRANDED ON THE NW SPANISH ATLANTIC COAST

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Abstract

In North Atlantic European waters, the nasal mite *Halarachne halichoeri* has been described affecting Grey seals (*Halichoerus grypus*) producing different levels of respiratory disease. This study provides data on the prevalence, clinical signs and produced macro-pathology of this parasite mite infecting juvenile wild Grey seals stranded in North-Western Spanish coast. Among the 25 seals examined during the study, a total of 19 had nasal mites in their respiratory upper ways, including adult and larval stages. This represented a percentage of prevalence of 76±8'37. All the live positive seals presented a typical clinical symptomatology associated to upper respiratory tract infections. In dead positive seals, a light to intense sinusitis could be diagnosed macroscopically. The presence of the parasite in the nasal sinuses appears as the primary cause of the high respiratory tract symptomatology presented in most of the juvenile seals stranded in the north coast of Spain. Ultrastructural characterization by scanning electronic microscopy (SEM) has confirmed the taxonomic status of the mite. This identification of *H. halichoeri* represents the first description of the occurrence of this parasite in Southern Europe.

Key words

Halarachne halichoeri, grey seal, *Halichoerus grypus*, nasal mite, marine mammal, parasitism.

1. Introduction

Nasal mites of two Genera (*Orthohalarachne* Newell, 1947 and *Halarachne* Allman, 1847) have been reported in pinniped species as obligatory parasites of the upper respiratory tract. *Orthohalarachne* Newell, 1947 has been observed affecting Otariidae (sea lions and fur seals) and Odobenidae (walrus), while *Halarachne* Allman, 1847, is more commonly found in the nasal tract of Phocidae (seals) (Furman and Dailey, 1980; Geraci and St Aubin, 1987) and sea otters (Kenyon et al., 1965). In North Atlantic european waters, *Halarachne halichoeri* have been described to produce different levels of respiratory disease affecting Grey seals (*Halichoerus grypus*) (Baker, 1987, Baker et al.,1998). In the past few years, strandings and sightings of grey seals in the North-Western Iberian Peninsula (Spain) waters have increased considerably (López et al., 2002), expanding the normal range of distribution attributed to this seal species (e.g. Härkönen et al., 2007). The presence in this area, as well as strandings of juvenile individuals is regularly reported during the winter months (López et al., 2002).

This study provides data on the prevalence, clinical signs in live individuals and lesions found on necropsies in dead ones, of a parasite mite of the genus *Halarachne* infecting wild Grey seals stranded in NW Spain coast. Furthermore, ultrastructural identification of the main specific morphological characters of this parasite by means of scanning electronic microscopy (SEM) has been described for the first time.

2. Material and methods

From 1999 to 2009, a total of 38 stranded juvenile Grey seals have been attended by the Galician (North-Western Spain) Marine Mammal Strandings Network (CEMMA). When live juveniles presented health problems, they were transported to rehabilitation facilities for an adequate treatment to their pathologies. Complete physical examination was performed and special attention put to identify the presence of mites. The presence or absence of associated upper respiratory symptomathology was also recorded in clinical reports and nasal mucus was recovered when was present. The seals found stranded dead or that died during the rehabilitation attempts, were fully necropsied and sampled following standard protocols (Geraci and Lownsbury, 1993; Dierauf, 1994). During the necropsies, the lower jaw was removed to allow a clear inspection of the pharynx (oral and laryngeal parts). Furthermore, the skull was opened by its sagittal plane with a saw in order to exam the interior of the nasal cavities (sinuses) and passages (turbinates), nasal posterior aperture (choana) and nasal part of the pharynx (nasopharynx). This procedure was performed in all dead seals, except the first four ones, which were examined in detail through the anterior (nostrils) and posterior (choana) nasal apertures. The lower respiratory tract (trachea, bronchi and lungs) was also completely examined following the standard protocols of necropsy. Presence or absence of mites was recorded in a complete necropsy sheet, where all the macroscopic lesions observed during this procedure were also included.

In 16 cases of the 38 attended, the juvenile Grey seals were found dead, making it possible to perform a necropsy and complete sampling only to 5 fresh individuals. In 22 cases, the seals were still alive and 2 of them were immediately released after a general inspection. The remaining 20 were transported to rehabilitation facilities. All the cases involved juvenile animals (less than 130 centimetres of total length). Among the seals admitted to the rehabilitation centre, 5 individuals died during the rehabilitation attempt and were also fully necropsied and sampled. The remaining 15 were successfully rehabilitated and released. So, in this study, we have finally included 25 juvenile Grey seals; 15 alive and 10 dead.

Demographic parasitic values (prevalence and intensity of infection) were defined in accordance with the recommendations of Bush *et al* (1997). Intensity of infection was specified by means of a semi-quantitative methodology: (-) no parasites observed, (+) a few to moderate (1-20) number of mites; (++) intense number of mites (>20). This range was established according to Fay and Furman (1982), as they considered heavier infestation than 20 mites did appear to be sufficient to cause significant irritation. When possible, the parasites were collected and stored in alcohol 70% for further identification.

Diagnostic morphological studies were carried out following descriptions of Oudemans (1926) and Furman and Dailey (1980). Mites were examined by means of light microscopy (LM) following standard protocols. For ultrastructural diagnosis under scanning electron microscopy (SEM), 10 samples of adult and 10 samples of larval mites were fixed for 4 hours in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.3) at 4º C and washed for 30 minutes in the same buffer. Samples were then dehydrated in an ethanol series, critical point dried in CO_2 using a Polaron E3000, and sputter coated in a Polaron

SC500 using 60% gold/palladium. The samples were examined with a Philips XC30 SEM operated at 10-20 kV.

3. Results

3.1. Demographic parameters and localization

Table 1 summarizes the main results related to intensity of infestation and prevalence of *H. halichoeri* in the seals included in the study. Among the 25 juvenile seals examined, a total of 19 individuals had nasal mites in their respiratory upper ways. This represented a percentage of prevalence (P) of 76 ± 8,37. Among the positive seals, 13 (68,4%) presented light to moderate number of parasites (+), and 6 (31,6%) presented heavy mite infestations (++). In 2 of the seals, the total count of mites was over fifty (Figure 1). All dead seals were positive to the presence of mites. A complete examination of the upper respiratory tract was only possible in dead individuals during the necropsy, revealing that the number of parasite larvae was higher than adults. Mites were located at the nasal cavities and passages in all cases, but they were often observed migrating to nasopharynx. In both cases of intense infestation, mites were found coating the nasopharynx walls and choanal orifice (Figure 1). No mites were observed in the trachea, bronchial tree or lungs of any seal examined, nor in the oral cavity or the oesophagus.

3.2. Morphological identification of *Halarachne halichoeri*

Larval and adult mites were identified using a LM and SEM, and assigned to the *Halarachne halichoeri* species according to Oudemans (1926) and Furman and Dailey (1980). Adults (Figure 2A) were characterized by: body cuticle smooth with a prominent shield located at the anterior, idiosomal length up to 2800 µm (ranging from 2765 to 2830 µm; n=10) with subcylindrical form slightly constricted at anterior end of opisthosoma, dorsal shield 1200 µm (1178-1215 µm; n=10) long by 600 µm wide (595-607 µm; n=10), posterior end of dorsal shield linguiform and broader than anterior end, typical specific setae number and disposition at the anterior end of the body, and finally, femur of leg III and genu of leg IV with 6 setae.

Larvae (Figures 2B) were characterized by: bluntly elliptical idiosoma, 1200 μm long (ranging from 1185 to 1210 μm ; n=10) and 700 μm wide (696-703μm; n=10), legs with typical setae disposition according previous descriptions (Furman and Dailey, 1980), tarsi II and III with two claws large and sessile (Figure 2C), post-anal setae very long, 650 μm (648-654μm; n=10), and longer than adanal ones, 380 μm (376-382 μm; n=10) (Figure 2D), gnathosomal setae absent, and a small pair of hypostomal lobes medial to coxa-trochanteral joint of palpi.

3.3. Clinical signs and pathological effects

All the positive live seals presented a typical clinical symptomatology associated to upper respiratory tract infections; mucous or muco-purulent nasal discharge, sneezing and coughing trying to clear the nasal passages, facial pruritus and head shaking. Intensity of symptoms was correlated with the intensity of parasitation. In all dead animals examined, a light to intense sinusitis and rhinitis could be diagnosed macroscopically, characterised by mucous membrane congestion and fluid exudation. In 7/25 cases (28%), light to moderate lower respiratory tract lesions were diagnosed, including: congestion (4 cases), oedema (3 cases) and bronchopneumonia (6 cases). In 2 cases, bronchopneumonia was characterized by abundant muco-purulent exudates occupying main and secondary bronchi (catarrhal-purulent bronchopneumonia), while the other 4 cases were considered diffuse acute bronchopneumonias, affecting large areas of both lungs.

4. Discussion

The endoparasitic mesostigmatic mite group of the family Halarachnidae, subfamily Halarachninae, contains exclusively parasites of the respiratory tracts of mammals and consists of 6 genera and 34 accepted and described species (Furman and Dailey, 1980). One of these genera, *Halarachne*, occurs typically in members of the family Phocidae. The taxonomic validity of several significant characters used by Ouderman 1926 to the description of the species *Halarachne halichoeri*, and specifically those referred to the intraspecific measure variability, has been questioned by Furman & Dailey (1980), who clarify by LM some of those previously described characters. The application of SEM provides a means of defining more precisely the surface topographical features of species, although these features should also be used in combination with other identification methods. Nevertheless, no SEM studies have been carried out before on this species. The first SEM analysis described here allowed us to determine measurements, differential characters and setae disposition in adult and larvae mites examined in this work, that conform well to the description of *H. halichoeri* given by Furman and Dailey (1980).

The present study represents the first description of the occurrence of *H. halichoeri* in Southern Europe infecting the nasal tract of the Grey seal, widening their known geographical distribution. The high prevalence and intensity of infection recorded, as well as other descriptions in other Northern Europe areas (Baker, 1987; Baker et al., 1998; Rijks et al., 2008) seems to corroborate that Grey seals act as the most preferred host species, playing an important role in the life cycle of this endoparasitic mite in European waters.

Adult *Halarachne* have been commonly described in the nasopharyngeal and lung mucosa, while the larvae are found mainly in the nasal passages (turbinates), feeding on the mucosa (Fay and Furman, 1982). The life cycle includes a free-living hexapod, followed by octopod protonymph and deutonymph, and eventually by the adult. The hexapod larva is responsible for the propagation of infections since it is highly resistant and mobile. The protonymph and deutonymph have been described as stages with little activity and short-living (Newell, 1947). In our case, both adult and larvae individuals were found sharing the whole upper respiratory tract of the seals.

Generally, the number of *Halarachne* larvae is described higher than adults, which initially suggested a high mortality rate among the former (Kim et al., 1980; Fay and Furman, 1982). This ratio is coincident with parasite intensity and identification of both larval and adult forms described in our study. But on the other hand, this higher number of larval stages has also been observed in parasite species which similarly infest nasal cavities of terrestrial mammals, and different explanations have been raised. In Oestrid parasites of sheep, hypobiosis -an asynchronous slow development- of the larval stages allows them to survive overcrowding of too many larvae in a limited space (Angulo-Valadez et al., 2010). This arrested development also allows the larvae to prevent the immune responses of the host to the presence of adult parasites (Tabouret et al., 2003) and may also be considered a form of adaptation to the adverse winter climate conditions (Angulo-Valadez et al., 2010). All of these are consistent in our case, and so they have to be studied further in seal species.

Infestations by nasal mites have been mainly described in seals older than one year, but with highly variable incidence (Kim et al., 1980; Rijks et al., 2008). The severity of the infestation is also extremely variable and depends on the health status and age of infested individuals. In our case, all the affected seals were juveniles, most of them suffering from starvation and weakness, potentially related to the higher prevalence of *Halarachne* infestation. The transmission of the mites is by direct spread of larvae from seal to seal. Then, the high prevalence in juveniles we have found could be considered as a normal finding taking into account that they have recently had a close relationship mother-calf.

We also consider that the lower percentage of live individuals with heavy mite infestation (1/15, 6'7%) could be related to underestimation due to the impossibility of a complete parasite count in live seals. This underestimation is also possible in the case of the first four dead animals, due to the lack of opening the head by its sagittal plane during the necropsy, or because of the fact that some parasites may leave their host when it is dead. Both possibilities were largely minimized because the careful inspection through the nostrils in that seals and the fact that only recently dead seals were included in our study. Furthermore, in all of these four seals, parasites from the nasal cavity were observed and sampled, hence the results of prevalence are not affected and only intensity could be slightly underestimated.

Parasites have been implicated in disease processes of marine mammals as well as referred to as causes of stranding in marine mammals (Geraci and St. Aubin, 1987; Dierauf, 1990; Dailey, 2001). Nevertheless, in many instances it has been difficult to establish the link between morbidity and mortality of individuals and populations and exposure to parasites (Geraci and St. Aubin, 1987). The pathological action of nasal mites on pinniped host species has been much debated. They do not seem to cause severe trauma, in most instances. However, heavy nasal mite infections can produce enough damage to the upper respiratory tract to affect the health of a seal (Kim et al., 1980; Geraci and St. Aubin, 1987), and even Van Bree (1972) described the case of a Grey seal which died due to nasal obstruction by *H. halichoeri*.

Lower respiratory tract lesions such as pneumonia, oedema and lung congestion has been also described in seals which presented nasal mites in the nasal tract (Dunlap et al., 1972; Dailey, 2001). This fact do not suppose a compulsory relation, but in other mammal species such as the sheep, intense nasal parasitosis have been described to produce local recruitment and accumulation of eosinophils in other areas far from the infestation site, even in lower respiratory tract, which may produce lesions in the respiratory mucosa (Tabouret et al., 2003). In our study, 7/25 cases (28%) presented light to moderate lower respiratory tract disease. Although all these animals presented nasal mites in the upper ways, the respiratory problems associated with the lower tract could not be directly related with the mite infestation. Moreover, lesions such as inflammation of the nasal mucosa can also result in secondary bacterial nasopharyngitis, destruction of bony tissue (turbinates) or pulmonary affection (Mullen and OConnor, 2002), that could actually endanger the life of the affected seal.

Additionally, marine mammals are known to be highly bioaccumulative of pollutants since they are top predators, have long life span and a large amount of fatty tissues with tendency to accumulate contaminants (e.g. O'Shea, 1999; Reijnders et al., 1999; Das et al., 2003). Young seals generally arrive to NW Spain in a poor health conditions, and they are not habituated to handling, treatments, facilities, etc, becoming deeply stressed during the first days of rehabilitation. Causes such as high intake of bioaggressors and maintained stress situations have been described producing, directly or indirectly, impairment of immune responses to other common pathogens (virus, bacteria, fungi or parasites), and causing severe undercurrent disease outbreaks in wild marine mammal populations (O'Shea, 1999; Reijnders et al., 1999; Das et al., 1999; Kakuschke and Orange, 2007).

This immunosuppressant effect of pollutant bioaccumulation has been demonstrated in grey seal pups (Hall et al., 1997; 2003), and it has also been potentially related to mortalities caused by Phocine Distemper Virus (PDV). In northern Europe, PDV caused thousands of deaths among Harbour seals *(Phoca vitulina*) and Grey seals in 1988 and 2002. Among others, upper and lower respiratory symptoms and lesions were described in all the affected seals (Bergman et al., 1990; Härkönen et al., 2006; Rijks et al., 2008). In our study, 28% and 100% of the positive seals presented lower and higher respiratory tract affection, respectively, but to date, no PDV cases have been diagnosed among the seals attended in NW Spain. Therefore, the presence of the parasites in the nasal sinuses appears to be the primary cause of the high respiratory tract symptomatology presented in most of the juvenile seals stranded in this geographical area. The role of mass infestations of *H. halichoeri* should be studied further to determine its possible implication in facilitating the establishment of lower tract respiratory diseases.

Finally, some clinical and management recommendations should be inferred from the obtained data. Isolation is highly recommended for the individuals with upper respiratory tract symptoms, with the aim of avoiding the possibility of infestation seal to seal. It is also recommended the introduction of rhinoscopy (nasal scoping) in the diagnostic protocol, due to its demonstrated efficacy in other species to obtain direct images of the interior of the upper respiratory tract (McCarthy and McDermaid, 1990; Elie and Sabo, 2006; Pietra et al., 2010). Finally, when severe mite infestations are detected, antiparasitical drugs have to be administered in order to prevent the lesions and clinical signs described here. Ivermectine has been reported as useful agent against marine mammal mites at a subcutaneous dose of 200µg/kg, to be repeated in 2 weeks (Dailey, 2001).

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Table 1. Demographic parasitic (*H. halichoeri*) values of grey seals stranded in Galician coasts (NW-Spain).

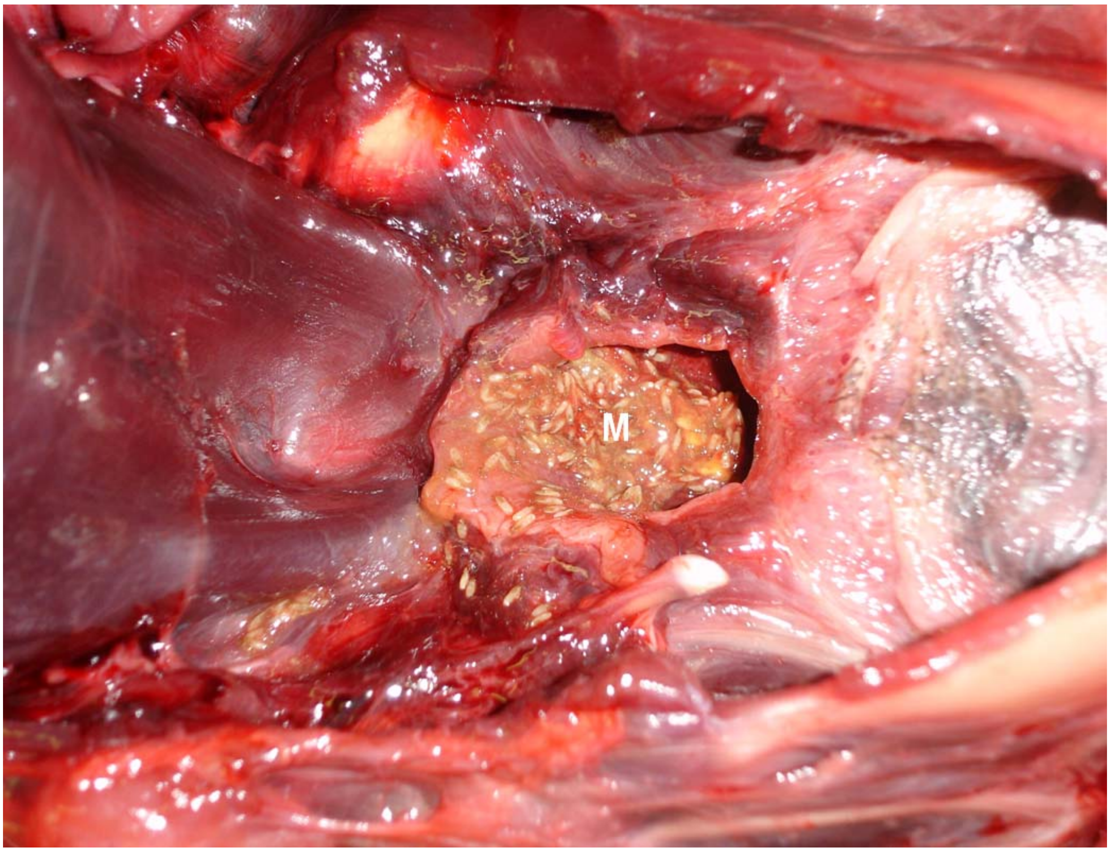
		-	+	++	Total
Alive	**N (%)**	6 (24)	8 (32)	1 (4)	15 (60)
Dead	**N (%)**	0 (0)	5 (20)	5 (20)	10 (40)
Total	**N (%)**	6 (24)	13 (52)	6 (24)	25 (100)
	P (% ± CI)	-	52 ± 9.79	24 ± 8.37	100 ± 0

- Semi-quantitative intensity of infection: (-) no parasites observed, (+) a few to moderate (1-20) number of mites; (++) intense number of mites (>20).
- N (%): number of seals (percentage from the total seals included in the study)
- P (%±CI): prevalence (percentage of parasitized seals from the total seals included in the study ± confidence interval)

Figure captions

Figure 1. Macroscopic aspect of the choanal orifice of a stranded grey seal with a heavy mite infestation (M). Right side towards the oral cavity, left side towards the larynx.

Figure 2. A): Scanning electron micrography of adult *Halarachne halichoeri;* general dorsal view showing a linguiform shield (Sh) and legs with the typical setae disposition. B): Scanning electron micrography of larvae *Halarachne halichoeri*; general ventral view of the bluntly elliptical idiosoma, leg disposition and typical setae distribution. C): Leg tarsi showing two large claws (Cl) and typical leg setae (white arrows) disposition. D): Detail of the post-anal setae (PAs) longer than adanals (As).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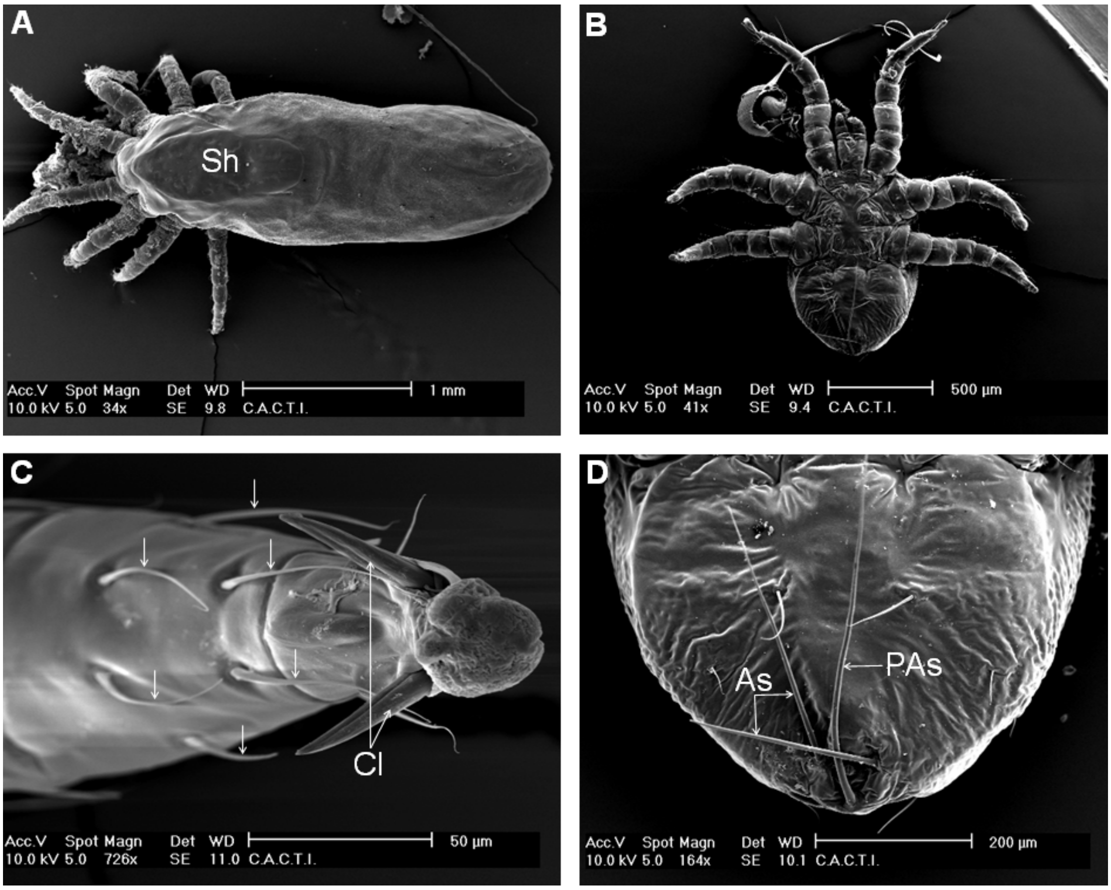
A
Sh
Acc.V Spot Magn Det WD 1 mm
10.0 kV 5.0 34x SE 9.8 C.A.C.T.I.
B
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10.0 kV 5.0 41x SE 9.4 C.A.C.T.I.
C
Cl
Acc.V Spot Magn Det WD 50 µm
10.0 kV 5.0 726x SE 11.0 C.A.C.T.I.
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10.0 kV 5.0 164x SE 10.1 C.A.C.T.I.