Aging Steller Sea Lions by Growth Layer Groups in Teeth

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Abstract

1 Accurate age determination of individuals is crucial in estimating age structure in populations as well as age-specific rates of reproduction and survival. We examined growth layer groups (GLGs) in the canine, incisor, and postcanine teeth of Steller sea lions (*Eumetopias jubatus*) to find the optimal method to determine their age using 18 known-age animals, from data collected in Hokkaido, Japan, 1995–2011. After checking to determine if the pulp cavity was open, nearly closed, or closed, teeth were sectioned and stained. Occlusion of the canine pulp cavity occurred at older ages than with the incisor and postcanine teeth.

Initially, the number of GLGs in both dentin and cementum was compared with actual ages to confirm the age determination method. Dentin GLGs appeared clearly in all of the canines, incisors, and postcanines. The number of GLGs was consistent with the actual age, with the exception of teeth with a closed pulp cavity. With respect to cementum, appearance of GLGs was rather obscure and no GLG was detectable at ages of 0 years and 1 year. Thus, the number of GLGs in cementum could be assigned as age when the value 1 year was added. We examined the accuracy and precision of the tested methods using an independent reader, who did not know the actual ages. By conducting readings 3 times, we used coefficient of variation (CV) to determine precision. The most precise CV was obtained in dentin canine (6.7%). Accuracy was calculated as proportion of estimates without error. The greatest accuracy in dentin was observed in canines (0.83, n = 18), whereas that of cementum was in postcanines (0.36, n = 11). We

concluded that canine dentin provided the most precise and accurate ages for Steller sea lions. Once closure of the pulp cavity in canines has occurred at the age of 15–16 years in males and 11–12 years in females, we recommend postcanine cementum be used.

Graphical Abstract

We examined growth layer groups in the canine, incisor, and postcanine teeth of Steller sea lions (*Eumetopias jubatus*) to find the optimal method to determine their age using 18 known-age animals. We concluded that canine dentin provided the most precise and accurate ages for Steller sea lions. Once closure of the pulp cavity in canines has occurred at the age of 15–16 years in males and 11–12 years in females, we recommend postcanine cementum be used.

KEY WORDS age determination, aging, cementum, dentin, *Eumetopias jubatus*, growth layer groups, Steller sea lion.

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Accurate age determination is essential to understand population dynamics, including age-specific rates of survival and pregnancy (Laws 1962, Hohn 2008). Counting tooth-growth-layer groups (GLGs)—comprising dark and light layers in dentin or cementum—is a common method for aging marine mammals that has been used since the 1950s (Scheffer and Myrick 1980). Dentin and cementum layers in canine teeth have been used to determine age in Steller sea lions (*Eumetopias jubatus*) since the early 1960s (Fiscus 1961, Spalding 1964). Permanent teeth already exist in the gums of Steller sea lions when pups are born but these do not erupt until their first autumn through winter (Spalding 1966, King et al. 2007).

Rather than premolars or molars, postcanine teeth are used for the nearly homodont condition of the cheek teeth in pinnipeds (King 1983). Within the dentin, a neonatal line appears at the birth, and then annual GLGs are formed inside the pulp cavity (Fiscus 1961). A GLG representing 1 year comprises a dark-opaque layer formed during winter and a light-translucent layer formed during early summer (Fiscus 1961). Deposition of GLGs continue until closure of the pulp cavity at approximately 15–16 or 11–12 years of age for male and female Steller sea lions, respectively (Fiscus 1961).

For cementum, the first growth layer appears at 15 months of age and annual GLGs deposit during August to September during and after 2 years of age for Steller sea lions (Spalding 1964). The upper third incisor (hereafter, incisor) and the second postcanine (hereafter, postcanine) have been used for age determination as well (Fiscus 1961, Pitcher and Calkins 1981, Calkins and Pitcher 1982). The dentin GLGs for 3 of the 4 incisors corresponded to those of the canine dentin (Fiscus 1961). Postcanine cementum GLGs were confirmed to deposit annually based on 9 marked animals (Pitcher and Calkins 1981, Calkins and Pitcher 1982). Such cementum age estimation has been confirmed for many other pinnipeds and individuals of known age (Childerhouse et al. 2004, Hohn 2008).

However, the efficacy of using GLGs as an age characteristic has not been confirmed for Steller sea lions whose actual age is known. Moreover, precision and accuracy of age determination based on tooth layers have not been described for

Steller sea lions. For instance, only 54% of the ages determined for adult Steller sea lions using tooth layers (n = 185) agreed between 2 independent examiners (Thorsteinson and Lensink 1962). Thorsteinson and Lensink (1962) determined ages by cutting each tooth through the midsagittal plane, polishing one of the halves without staining, and counting internal laminations. In contrast, we used animals marked as pups by hot-branding or plastic tags at Russian rookeries from 1989 to 2011 to study movements and demography (Burkanov et al. 2011, Permyakov et al. 2014). A total of 7,586 and 3,021 Steller sea lions were marked by hot branding and plastic-tags, respectively (V. N. Burkanov, Russian Academy of Sciences, unpublished data). Since 1995, some of the marked animals have been collected off Hokkaido Island, Japan, by incidental fishery mortality or damage-controlling culls. These specimens provided opportunities to evaluate the accuracy of age determination by comparing the age determined by GLGs with actual age of the animal.

Study Area

Specimens belonged to the Asian stock of the western Steller sea lions (*E. j. jubatus*), which were distributed on the continental shelves in the North Pacific, ranging from 144°W to Japan (Baker et al. 2005, Phillips et al. 2009). Although there were not breeding rookeries in Hokkaido Island, the area was used as wintering habitat. A previous study in Hokkaido found that individuals originated from the western section of the Asian stock, including the Kuril Islands and the northern Okhotsk Sea (Isono et al. 2010). The population of the Asian stock experienced a severe decline of 70% during the late 1970s and 1980s (Loughlin et al. 1992). However, the western section of the Asian stock has been recovering since the 1990s (Burkanov and Loughlin 2005).

Methods

We collected teeth from Steller sea lions in Hokkaido from January through May in 1995–2011. These animals were legally shot for fishing-damage control or incidentally caught in fishing nets used by Japanese fishermen. We examined upper canines, third incisors, and second postcanines from 18 marked individuals. Previous studies have used these teeth for age determination of Steller sea lions (Fiscus 1961, Calkins and Pitcher 1982). Sampling month can influence the appearance of latest GLG (Table 1). After boiling the skull for 1 hour at atmospheric pressure, we extracted the teeth. We classified the pulp cavity of each tooth into the 3 categories as 1) open, 2) almost closed (>80% closed), or 3) closed.

We prepared teeth sections based on Hachiya and Ohtaishi (1994) using Delafield's hematoxylin (Gilbert 1966). We sliced teeth mid-longitudinally into sections 5–8 mm thick using a diamond cutter (Meihan MPC-400, Gifu, Japan). Then we decalcified sections with Plank–Rychlo's solution (Plank and Rychlo 1952) for 48 hours. After neutralization by sodium sulfate solution for 12 hours, we rinsed them with running tap water. We sectioned specimens at 50 μm using a freezing microtome (Yamato Kohki Industrial NS-31, Saitama, Japan), which were then mounted on glass slides coated with gelatin. We stained sections using Delafield's hematoxylin in 30, 35, or 40 minutes in each tooth to observe the difference of contrast, and then rinsed in running tap water. We mounted sections by cover glasses with Bioleit (Okenshoji Co., Ltd., Tokyo, Japan) after dehydration by alcohol and xylene.

We observed sections of dentin and cementum using a microscope (Zeiss Stemi 2000-C, Oberkochen, Germany) at 5×–40× with transmitted light. Initially, correspondence between the number of GLGs and actual ages was observed to confirm age assignment from the number of GLGs and clarify the influence of the occlusion of the pulp cavity by age. Subsequently, another independent reader in another laboratory, who did not know actual ages, tested accuracy and precision of age assignment. We choose the best section in each tooth of each animal, with these sections randomly selected for readings. Readings were conducted by blind review 3 times without referring either to the actual age or the results of the previous readings. When 2 of the 3 readings agreed to an age, we assigned that age to the tooth. If all 3 readings did not agree, we used the mean value of the 3 as long as the disagreements were within the range of 2 years. If the disagreements were ≥3 years, we reread sections until we assigned an age as above (Dickie and Dawson 2003, Childerhouse et al. 2004). We assigned only one reader who was well-skilled with an experience of aging >100 Steller sea lions to minimize possible bias between readers (Oosthuizen 1997). To determine precision of readings, we used coefficient of variation (CV; Chang 1982, Campana 2001):

$$CV_j = 100\frac{\sqrt{\sum_{i=1}^{R}\frac{\left(X_{ij}-X_j\right)^2}{R-1}}}{X_j}$$

where X_j and X_{ij} is the age of the *j*th animal determined in the *i*th time and *R* is the number of times each animal was aged. Although no reference point has been defined for CV of age determination in mammals, CVs <7.6%, have been acceptable in aging studies of fish (Campana 2001). We excluded animals 0 years both of actual age and assigned age from the calculation of CV because the denominator in the formula did not allow 0. To compare CV between the 3 types of teeth, we performed an analysis of variance using the software package R (R Core Team 2017). We calculated accuracy as the proportion of the estimated ages with differences from the true ages.

ResultS

The age of animals whose teeth were sampled ranged from 0 to 11 years, and averaged 3.9 ± 3.0 years in females (±SD) and 3.0 ± 3.5 years in males (Table 1). The pulp cavity of the canine was open in all individuals (Table 1). Although no incisor was available from males 6–10 years old, the pulp cavity was closed in an 11-year-old male. The incisors pulp cavity in females with an actual age of 8 years old were almost (n = 1) or completely (n = 1) closed. The pulp cavity of postcanines was almost closed at 5 years of age and completely closed at ages of ≥8 years in both sexes.

The dentin GLGs stained for 40 minutes showed slightly greater contrast and distinctness of GLGs than those stained for 30 minutes. A thin, but clear transparent line appeared, then deposition of dentin GLGs continued. The dentin GLGs in canines comprised alternative series of wide, dark-colored layers and narrow, light-colored layers (Fig. 1). The former and latter appeared as navy blue and white bands on the stained section, respectively. Of these, light-colored layers were counted as annual growth lines. Similar appearance of dentin GLGs and the transparent line were observed in incisors and postcanines. The number of dentin GLGs in canines was consistent with the actual age for all of the 18 animals (Fig. 2). Of these, one animal sampled during May represented a light-colored growth layer on the outer edge, while others (n = 17) were surrounded by a dark-colored layer (Fig. 3). With respect to incisors and postcanines, dentin GLGs in one animal were obscure, so this individual was excluded from the analysis. The number of dentin GLGs agreed with actual ages in 15 of 17 animals. Disagreements occurred in the specimens whose pulp cavities had closed (ages 8 and 11; Fig. 2).

Although GLGs appeared clearly in dentin, the GLGs in cementum were obscure and only partially readable. The cementum GLG pattern consisted of a thin, stainable layer and a thick, weakly stained layer (Fig. 4). Of these, the number of thin, stainable layers was counted as an indicator of age. The cementum GLGs stained for 30 minutes were obscure while those stained for 40 minutes were distinguishable. No

GLG was visible in cementum of canines from age 0- and 1-year animals. The magnitude of disagreement between the number of cementum GLGs and actual age ranged between 0 and 4 for age ≥2-year-old animals (Fig. 5). These disagreements averaged at 1.1 ± 1.0 year, 1.4 ± 1.4-year, and 1.1 ± 1.0-year for canines, incisors, and postcanines, respectively (mean ± SD). The deposition of the latest growth layer in cementum was undetectable because of the thick and dark-colored outer edge.

The mean of dentin CV was 6.7% in canines, 7.1% in incisors, and 8.5% in postcanines. No difference was detected by type of tooth in CV ($F_{2, 39}$ = 0.17, P = 0.84). For cementum, the mean CV was similar among type of tooth: 18.2% in canines, 13.6% in incisors, and 17.9% in postcanines, ($F_{2, 29}$ = 0.11, P = 0.90). Accuracy derived from counting dentin GLGs (n = 18) was 0.83 in canines, 0.72 in incisors, and 0.61 in postcanines. To assess the accuracy of aging using cementum, we added the value 1 to the number of GLGs. The teeth from the animals at the ages of ≤1 year were excluded because no GLG was detected in both ages. Accuracy in cementum GLGs was 0.27 (n = 11) in canines, 0.20 (n = 10) in incisors, and 0.36 (n = 11) in postcanines.

Discussion

The number of dentin GLGs agreed with actual ages when teeth with open pulp cavities were used, whereas disagreements occurred in teeth with closed pulp cavities. This inconsistency has been considered to be due to cessation of dentin deposition following closure (Oosthuizen 1997, Childerhouse et al. 2004). Closure of the pulp cavity was found in postcanines and incisors for ≥8-year-old and ≥5-year-old animals, respectively, whereas no closure was observed in canines in the present study. The pulp cavity in canines was mostly or completely closed at the age of 15–16 years in males and 11–12 years in females (Fiscus 1961). Thus, dentin GLGs in canines can be used up to these ages as an age determinant. Interestingly, body length and body mass in Steller sea lions attain asymptotes at these ages (Winship et al. 2001).

Sampling dates of the specimens used in the present study ranged from 9 January to 22 May. Of these, only specimens sampled on the latest date (i.e., May 22) represented a growth layer on the edge of dentin. This suggests that the deposition of dentin growth layer occurs during or after the last half of May, which agrees with Fiscus (1961), who reported the deposition of growth layers during early summer. Steller sea lions pups are born from late May to early July (Calkins and Pitcher 1982); therefore, the number of dentin GLGs represents the age when specimens were collected, except in summer.

The difference between actual age and cementum GLGs was approximately 1, indicating that the first GLG forms at the age of 2 years and then GLGs deposit annually. The obscurity of cementum GLGs in age 0- and 1-year animals encountered in the present study also support this conclusion. However, previous studies found that the first cementum GLG appeared at the age of 15 months (late summer of first yr) in Steller sea lions (Spalding 1964). Thus, when animals died from late summer to next birth day (i.e., averaged in June; Calkins and Pitcher 1982), 1 year should be added to the cementum GLGs counts. Cementum GLGs formed during ages of 0 and 1 in this study were far less discernible when compared with dentin GLGs; it has also been shown that the cementum layer in young teeth usually is not thick enough to reveal the annual lines clearly (Fiscus 1961). Mechanical stress, such as dental biting, is usually required for the deposition of cementum, unlike dentin (Klevezal 1996). But Steller sea lions often have an extended lactating period (≥12 month: Pitcher and Calkins 1981, Trites et al. 2006). This suggests that less mechanical stress in pups 0–1 year of age, which could result in a lack of clear cementum deposition.

The methodological development for age estimation using tooth annuli has focused on how to ensure that age estimates are both accurate and precise (Hohn 2008). Our results indicate that by counting GLGs in dentin, one can obtain better precision when compared with other teeth and cementum. More skilled readers or relatively easy age determination results in better precision (Campana 2001, Gunn et al. 2008). The reader assigned in the present study was well-skilled, with experience aging >100 Steller sea lions using Carazzi's hematoxylin, which resulted in poorer staining of GLGs than occurred with our method. Canine-dentin GLGs are generally easier to be read than other teeth because of the greater size of the tooth, with resulting wider spacing between annuli (Oosthuizen 1997). Although the precision of age determination for marine mammals has not been evaluated thoroughly, the precision value in dentin for Steller sea lions (6.61–7.96%) was lower than for sperm whales (*Physeter macrocephalus;* CV = 10.6%; Evans et al. 2002) and polar bears (*Ursus maritimus;* CV = 15.2% and 18.2%; Christensen-Dalsgaard et al. 2009).

The most accurate result was obtained by counting GLGs in canine dentine (0.83). In previous pinniped studies, cementum had been used because the occluded pulp cavities prevent accurate age determination in dentin (Childerhouse et al. 2004, Hohn 2008). However, the most accurate means for age determination in Steller sea lions is with animals before they attain the growth asymptote at approximately age ≤15 years for males and ≤10 years for females. Greater accuracy of dentin GLGs, compared with cementum, was also found in Cape fur seals (*Arctocephalus pusillus;* Oosthuizen 1997). Once closure of the pulp cavity in canines has occurred at the age of 15–16 years in males and 11–12 years in females (Fiscus 1961), we recommend using GLGs in postcanine cementum to determine age because of the greater accuracy among teeth. Note, however, that 1 year should be added to the cementum GLGs counts for Steller sea lions.

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Figure

Figure 1. Example of dentin growth layer groups (GLGs) in canines obtained from Steller sea lion male (sampled in Hokkaido, Japan, during 2010) with known age of 11.8 years (ID number 10401 in Table 1). In the pictures, GLGs are marked. Sections were obtained by cutting teeth longitudinally, decalcified, sectioned into 50 μm, and stained by Delafield's hematoxylin.

Fig. 1

Figure 2. Relationship between the number of dentin cementum growth layer groups (GLGs) and actual age of Steller sea lions (*n* = 18) collected in Hokkaido, Japan, 1995–2011. With respect to incisors and postcanines, dentin GLGs in one female were excluded from this observation because of section obscurity. This comparison was conducted to confirm the age determination method before examining the accuracy and precision of the tested methods using an independent reader, who did not know the actual ages. Solid lines represent 1:1 equivalent line.

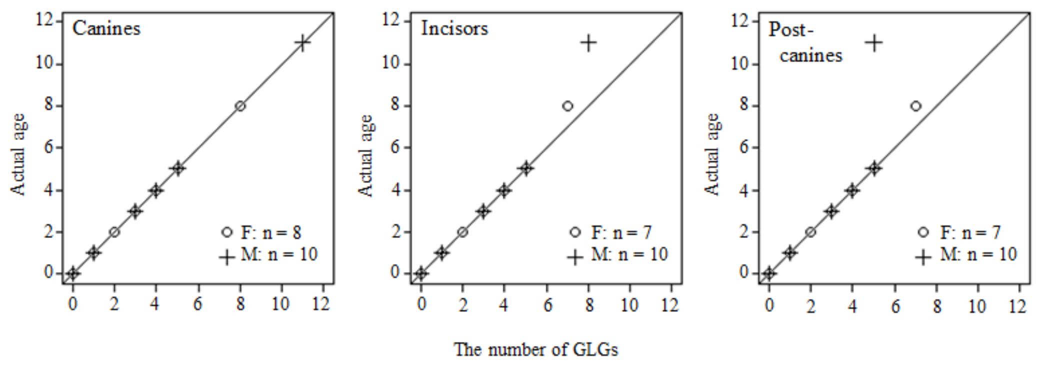

Fig. 2

Figure 3. Example of newly added outer growth layer in canine dentin from Steller sea lion male (sampled on 22 May 2010 in Hokkaido, Japan) with known age of 0.9 year (ID number 10212 in Table 1).

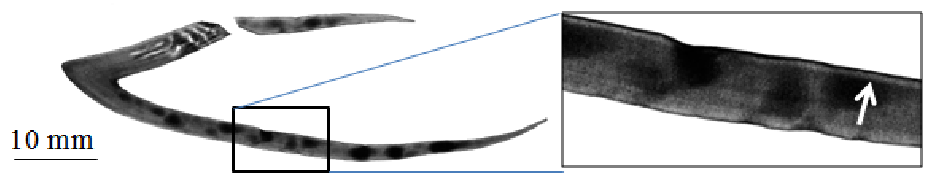

Fig. 3

Figure 4. Example of cementum growth layer groups (GLGs) in canine from Steller sea lion male (collected in Hokkaido, Japan, during 2010) with known age of 2.8 years (ID number 10103 in Table 1). In the pictures GLGs are marked. Sections were obtained by cutting teeth longitudinally, decalcified, sectioned into 50 μm, and stained by Delafield's hematoxylin.

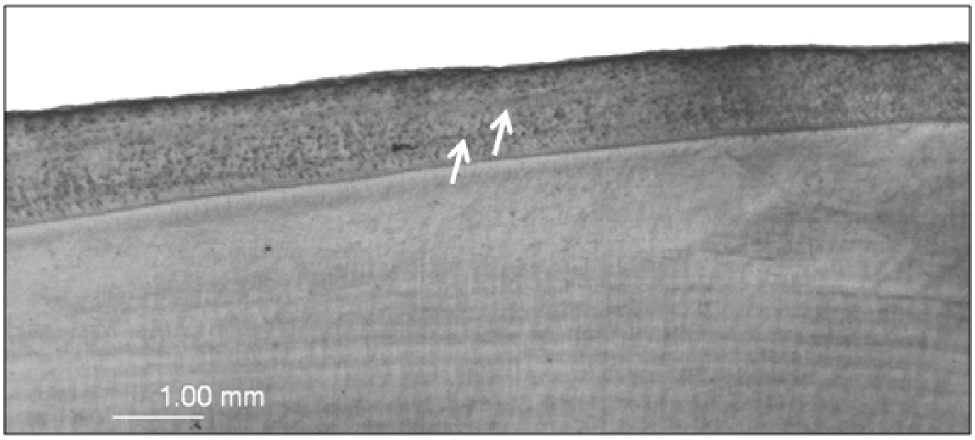

Fig. 4

Figure 5. Relationship between the number of cementum growth layer groups (GLGs) and actual age of Steller sea lions (n = 18). In each tooth, GLGs were obscure in females (ID number 05001 in canine, 06109 in Incisor, and 05108 in Postcanine); we excluded these animals from this observation. This comparison was conducted to confirm the age determination method before examining the accuracy and precision of the tested methods using an independent reader, who did not know the actual ages. Solid lines represent 1:1 equivalent line.

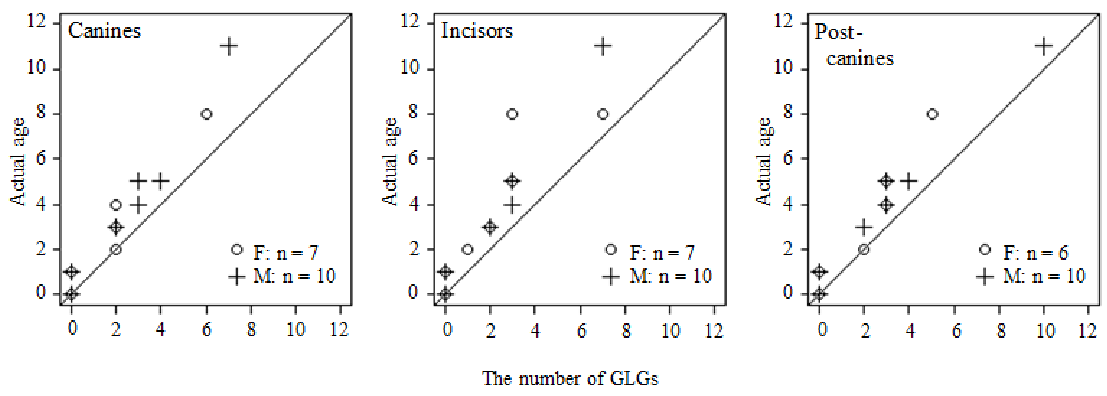

Fig. 5

Table 1. Information on degree of pulp cavity closure of marked Steller sea lions collected in Hokkaido, Japan, 1995–2011.

ID number	Sex	Age	Date of collection	Place of collection	Branded ID number (Plastic tag)	Incisor	Canine	Postcanine
07205	F	0	28 Mar 2007	Rebun	Ir717	○	○	○
05005	F	1	9 Jan 2005	Rausu	P790	○	-	○
10103	F	2	30 Mar 2010	Kamoenai	Br971	○	○	○
05108	F	3	25 Feb	Kamo	P657	○	-	○

			2005	enai				
06109	F	4	10 Mar 2006	Kamoenai	Ir318	○	-	○
05001	F	5	8 Jan 2005	Rausu	Br556	○	-	●
98008	F	8	24 Jan 1998	Nemuro Strait	Br85	●	○	●
98011	F	8	25 Jan 1998	Nemuro Strait	Br8	●	○	●
06112	M	0	12 Apr 2006	Otaru	Y553	○	○	○

10211	M	0	18 May 2010	Rebun	Gr156	○	○	○
10212	M	0	22 May 2010	Rebun	Gr34	○	○	○
97015	M	1	1 Feb 1997	Nemuro Strait	(P380)	○	○	○
05401	M	1	6 Apr 2005	Ofuyu	Y102	○	○	○
11102	M	3	25 Jan 2011	Iwanai	Br913	-	-	-
06110	M	4	17 Mar 2006	Iwanai	Ir278	○	-	○

95012	M	5	8 Feb 1995	Rausu	Br99	○	○	●
95101	M	5	4 Mar 1995	Kamo enai	C141	○	○	●
10401	M	11	1 Apr 2010	Ofuyu	Ir190	●	○	●

○: pulp cavity was opened, ●: nearly closed (>80% is closed), and ●: closed.

-: missing