

## Original Article

**A micro-computed tomographic evaluation of maxillary first molar root canal morphology in Black South Africans**Casper H. Jonker<sup>\*1,2)</sup>, Ericka N. L'Abbé<sup>2)</sup>, Peet J. van der Vyver<sup>3)</sup>, Daniel Zahra<sup>4)</sup>, and Anna C. Oettlé<sup>5)</sup><sup>1)</sup>Faculty of Health, Peninsula Dental School, University of Plymouth, Truro Dental Education Facility, Knowledge Spa, Royal Cornwall Hospital, Truro, UK<sup>2)</sup>Department of Anatomy, Forensic Anthropology Research Centre, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa<sup>3)</sup>Department of Odontology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa<sup>4)</sup>Peninsula Medical and Dental Schools, Faculty of Health, University of Plymouth, UK<sup>5)</sup>Anatomy and Histology Department, School of Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa

Received February 25, 2024; Revised April 18, 2024; Accepted April 20, 2024; J-STAGE Advance Publication: June 8, 2024

**Abstract****Purpose:** This study was conducted to investigate the root canal anatomy of maxillary first molars in Black South Africans.**Methods:** Micro-computed tomography was used to investigate 101 maxillary first molars (53 teeth from the right, 48 from the left; 50 male and 51 female teeth). The number of root canals in each tooth was determined, and the relationship between side, sex and age was analyzed using chi-squared test. To determine intra- and inter-observer reliability, Cohen's kappa coefficients were calculated.**Results:** Intra- and inter-rater agreements of 96.92% and 98.08% were achieved, respectively. Most teeth contained either three or four canals, but a second, third and fourth mesio-buccal canal was found in 60.39%, 5.94% and 0.99% of teeth, respectively. The disto-buccal and palatal roots contained predominantly single canals, but additional canals were noted in 2.97% and 1.98% of teeth. Four canals were common in females and teeth on the right side often contained a second mesio-buccal canal. However, the prevalence of a third mesio-buccal canal was higher in males than in females.**Conclusion:** The teeth studied showed diversity and variations between sexes and arch sides. These findings will aid clinicians in endodontic treatment and will be applicable for educational purposes.

Keywords: additional canals, C-shaped canals, micro-CT, number of canals, second, third and fourth mesio-buccal canals

**Introduction**

Endodontics involve a series of mechanical and chemical steps to remove irreversibly inflamed or infected tissues from root canal spaces. To ensure the best possible outcome, all root canals must be discovered and treated [1]. The success rate of molar root canal treatment can be as high as 91.7% but despite a clinician's best efforts, treatments can still fail [2]. Possible reasons for treatment failure include complex root canal anatomy and undiscovered root canals. Untreated areas within the root canal system can harbour infected organic material, which can jeopardize treatment success [1]. Root canal morphology also differs across populations [3].

The maxillary first molar has been identified as one of the most challenging teeth to treat due to its pulpal complexity and presence of additional canals [4]. A missed second mesio-buccal canal (MB2) located in the mesio-buccal (MB) root has been identified as a common cause of treatment failure, as it can be difficult to locate and treat [1]. The prevalence of an MB2 canal has been estimated to range between 48% and 97.6% of individuals [5]. In addition to the MB2, a third mesio-buccal canal (MB3)

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or additional canals in the disto-buccal (DB) and palatal (P) roots can also be present [4,6]. Currently, there are no available reports detailing micro-computed tomography (micro-CT) studies of maxillary first molar root canals in Black South Africans.

Micro-CT is a non-destructive and accurate method for evaluation of root and canal morphology in three dimensions at high definition. In 1995, Nielsen et al. [7] were the first to describe a maxillary first molar in high definition using micro-CT technology. This technology has since become popular for visualization of complex root canal anatomy in fine detail [8]. The aim of the present study was to provide information on root canal morphology and variants found in maxillary first molars of Black South Africans.

**Materials and Methods**

The study design was quantitative, descriptive, cross-sectional and observational and reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [9] (Fig. 1).

**Origin of scans**

Human skulls, with known age, sex and population affinity, were sourced from the Human Osteological Research Collection (HORC) housed in the Anatomy and Histology Department of the Sefako Makgatho Health Sciences University (Pretoria, South Africa) and the Pretoria Bone Collection (PBC) housed in the Department of Anatomy of the University of Pretoria (South Africa) [10]. Once permission for research has been given by family members, these bodies form part of the whole body donation program. The Director General grants approval for unclaimed bodies to be used for research purposes. In such cases, samples are protected by the National Health Act 61 of 2003 [Republic of South Africa. National Health Act 61 of 2003. Government Gazette No. 26595 (updated 23 July 2004; cited 3 December 2023)] in the case of unclaimed bodies.

The skulls were scanned at the South African Nuclear Energy Corporation (Necsa, Pelindaba, South Africa) using a Nikon XTH 225L industrial CT system (Nikon Metrology, Leuven, Belgium) incorporating a micro-focus X-ray computed tomography scanner, as part of the Bakeng se Afrika, Erasmus+ capacity building project. The following operating parameters were adopted: 100 kV voltage, 100 mA current and 2.00 s exposition time per projection. The spot size of the X-ray unit is between 0.001 and 0.003 mm (1-3  $\mu$ m). The unit contains a Perkin Elmer detector with a 400 mm  $\times$  400 mm field of view and a pixel size of 200  $\mu$ m  $\times$  200  $\mu$ m [Hoffman JW, De Beer FC Characteristics of the Micro-Focus X-Ray Tomography Facility (MIXRAD) at Necsa in South Africa. 18th World Conference on Nondestructive Testing, Durban, South Africa, 16-20 April, 2012]. Following the scanning process, the two-dimensional (2D) projection images were reconstructed with Nikon CT Pro version 4.4.3 software (Nikon Metrology) into the final three-dimensional (3D) volumes. Isotropic voxel sizes/resolution of scans ranged between 40  $\mu$ m and 62  $\mu$ m. Once the final volumes had been obtained, the final 3D volumes were imported into Avizo 2019 (Visualization Sciences Group Inc. Bordeaux, France), a 3D visualization program, for the subsequent post-acquisition processes [Westenberger P. Avizo – Three-Dimensional Visualization Framework. In Proceedings of the Geoinformatics – Data to Knowledge Conference, Potsdam, Germany, 11-13 June, 2008].

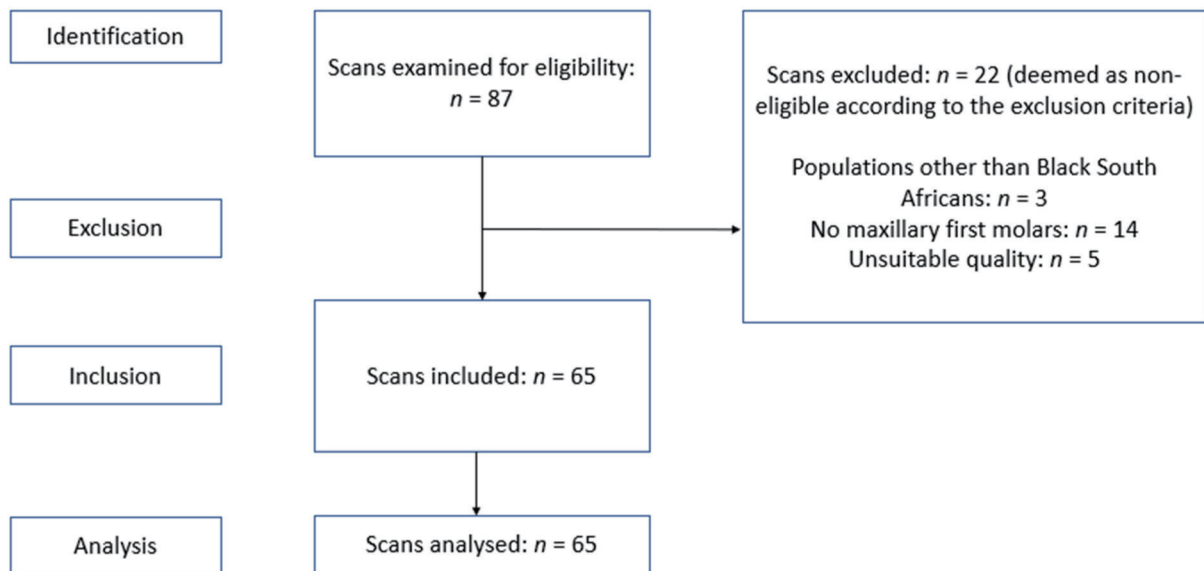


Fig. 1 STROBE flow diagram to indicate the eligibility of available scans

### Segmentation, alignment and image acquisition

Each micro-CT scan was opened and rendered in 3D using the Isosurface module within the Avizo 2019 software. Each maxillary first molar was then virtually extracted by cropping and segmenting the scan. Landmarks were placed on each tooth in the arch of the maxilla using the cemento-enamel junction (CEJ), commonly used as an ideal plane of reference [11]. For molars, a landmark was placed on the buccal and palatal surfaces at the highest occlusal point of each root along the CEJ, thus comprising three landmarks in total. From these landmarks, a best-fit plane was automatically computed at the level of the CEJ, which was used as a reference to re-align the micro-tomographic image stacks. These steps reduced the introduction of oblique sections and possible bias.

To allow virtual extraction and 3D observation of each component of a tooth (enamel, dentin, and pulp), a region-based semi-automatic segmentation procedure known as the watershed method was carried out [12]. Segmentation is a process in which each tooth and its inner components are isolated virtually from each other. Different colors were allocated to allow proper differentiation between components. Minor dentinal cracks were removed through a process known as masking (multiple-slice editing) [13]. Scans showing severe dentinal cracks were discarded.

### Scan analysis

Interpretation of scans was based on steps described by Fernandes et al. [14]. Observation of root canals and data collection were completed first by the main researcher, who is an experienced clinician in the field of endodontics with proficiency in Avizo to perform 3D imaging analyses of micro-CT images. Unique codes were allocated to each scan and data capturing was completed blind without prior knowledge of sex, side or age. To allow proper observation of root canals, the pulps from each scan were isolated from the other components, magnified and rotated using settings and parameters within Avizo. By adjusting the brightness, contrast and sharpness parameters, optimal imaging was achieved. The same parameters were used for each scan.

A second researcher and specialist in prosthodontics was approached to participate in inter-observer reliability determination. Prior to this test, both researchers were calibrated by observing images and reporting their findings for two randomly chosen scans unrelated to the main reliability test. During the main test, the researchers recorded their results independently, after which the results were compared. Any agreements were accepted, but any findings that differed were discussed until a consensus was reached. Intra-rater reliability was also confirmed by repeated observation of the same selection of scans by the main researcher within a one-week period [15].

### Inclusion criteria

Teeth from Black South Africans with intact roots and fully developed apices were considered. Scans of adequate quality (highest possible resolution) where the pulpal space could be adequately isolated were considered.

### Exclusion criteria

The following were excluded in this study: teeth with incomplete root formation, root fractures, coronal or radicular resorption, previous root canal treatments, decay obscuring any root canals, metal restorations, scans of poor quality and individuals from other self-identified population groups.

### Sample size

A convenience sample was used, and all available scans were considered ( $n = 87$ ). A total of 101 maxillary first molars from 65 scans were identified after consideration of the inclusion and exclusion criteria. Teeth from both sides of the arches (left and right) were included as well as males and females of different ages.

### Statistical analysis

R Statistical Software version 4.1.1 (R Core Team 2021.R. A language and environment for statistical computing. R Foundation for statistical computing. Vienna, Austria) was used. The variables in the study (arch sides, sex, and age) were analyzed using the chi-squared test of association ( $P < 0.05$ ). Chi-squared test was also used to analyze relationships between age and total number of canals, canals in each root, and presence or absence of MB2, MB3 and MB4 canals. To determine intra- and inter-observer reliability, Cohen's kappa coefficients were calculated. The percentage of agreement was determined by evaluating images of randomly selected scans for approximately 20% of the sample size ( $n = 20/101$ ).

## Results

### Sample characteristics and examiner agreement

The sample included 53 teeth from the right side ( $n = 53/101$ , 52.48%) and 48 from the left ( $n = 48/101$ , 47.52%), as well as 50 from males ( $n = 50/101$ , 49.5%) and 51 from females ( $n = 51/101$ , 50.5%). The ages of the individuals ranged between 20 and 70 years (mean: 41.84 years). Intra- and inter-rater reliability was determined to be high, with 96.92% and 98.08% agreement and kappa values of 0.94 and 0.96, respectively.

### Number of canals

The number of canals was calculated for each tooth, which included observation of sex and sides. Most teeth contained either three or four canals, but five or six canals were also noted (Fig. 2). The average prevalence of molars with three and four canals was 39.6% ( $n = 40/101$ ) and 50.49% ( $n$

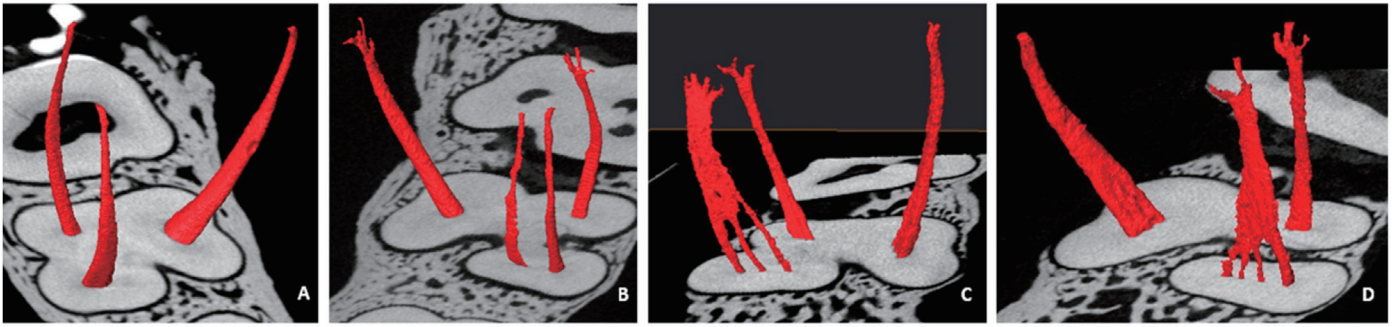


Fig. 2 (A) Three main canals, (B) Four main canals, (C) Five main canals, (D) Six main canals

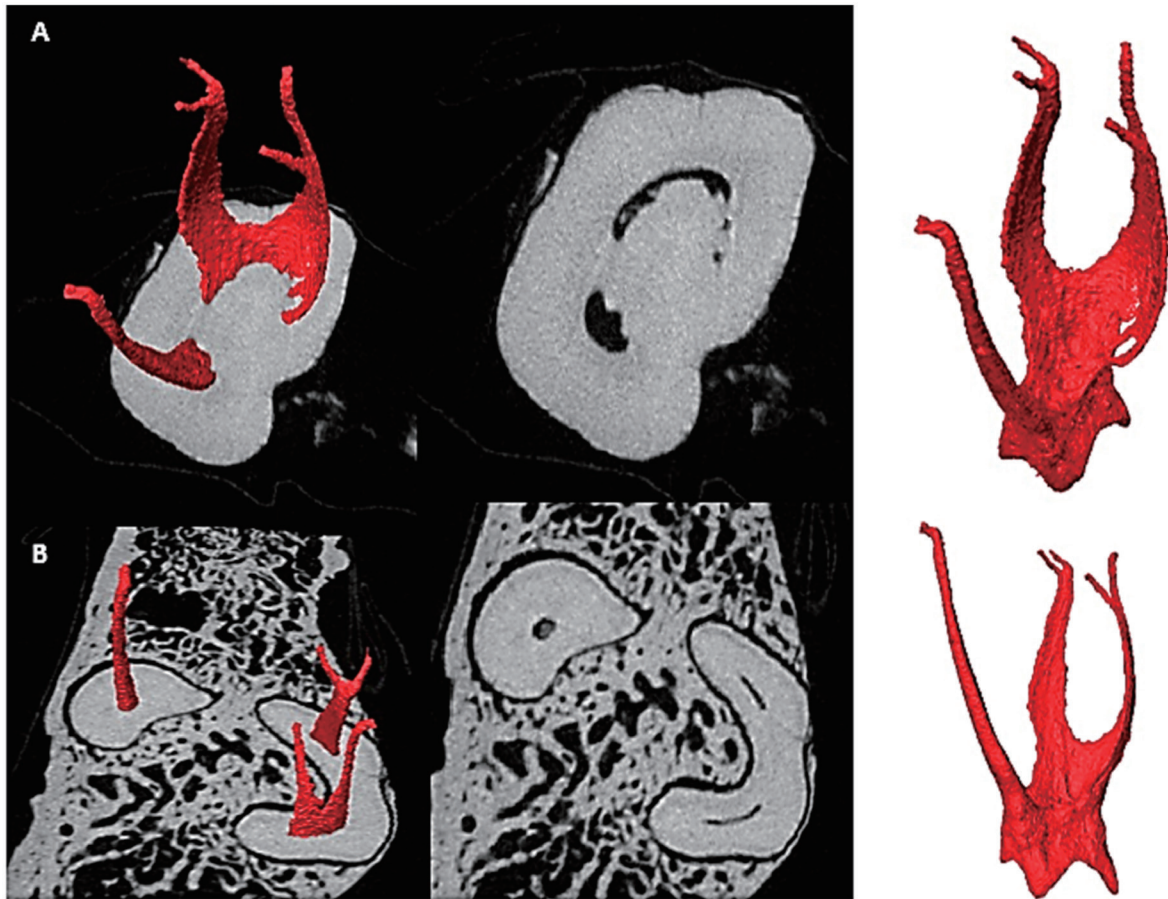


Fig. 3 (A) C3b type configuration [16] at orifice level, (B) C3a type configuration [16] in the apical third

= 51/101), respectively. The mean number of canals was 3.73 (SD: 0.72; median: 4; range: 3.00 and 6.00). The prevalence of five canals was 6.93% ( $n = 7/101$ ) and that of six canals 2.97% ( $n = 3/101$ ). In one tooth ( $n = 1/101$ , 0.99%), the coronal aspect had a C-shaped configuration which divided into separate canals in route to the apex. At the orifice level, this tooth had a C3b type configuration that changed to C3a in the midroot to apical third according to the classification of Fan et al. [16] (Fig. 3). No teeth contained one, two or more than six canals.

Teeth with three canals were similar for both males ( $n = 20/101$ , 40%) and females ( $n = 20/101$ , 39.22%) but four canals were more common in females ( $n = 29/101$ , 56.86%) than in males ( $n = 22/101$ , 44%). Teeth with five or six canals were more common in males ( $n = 6/101$ , 12% and  $n = 2/101$ , 4% respectively). On the left side, more teeth had three canals ( $n = 21/101$ , 43.75%) than the right side ( $n = 19/101$ , 35.85%). Teeth with four canals were more common on the right side ( $n = 29/101$ , 54.71%) than on the left ( $n = 22/101$ , 45.83%).

#### Number of canals per root

Findings are summarized in Table 1. In most MB roots, two canals were present. The mean number of MB canals was 1.67 (SD: 0.62; median: 2; range: 1-4). A single canal and two canals were found in 39.60% ( $n = 40/101$ ) and 54.46% ( $n = 55/101$ ) of MB roots. A single canal was found in most DB roots ( $n = 98/101$ , 97.03%) (mean: 1.04; SD: 0.24; median: 1; range: 1-3) and P roots ( $n = 99/101$ , 98.02%) (mean: 1.02; SD: 0.14; median: 1; range: 1-2).

#### Second, third, and fourth MB canals: MB2, MB3, and MB4

Multiple canals (two or more) were present in 60.39% ( $n = 61/101$ ) with a higher prevalence in males ( $n = 36/50$ , 72%) than in females ( $n = 32/51$ , 62.74%). The prevalence of MB2 and MB3 was 60.39% ( $n = 61/101$ ) and 4.95% ( $n = 5/101$ ), respectively. More teeth on the right side had MB2s ( $n = 34/53$ , 64.15%) than those on the left ( $n = 27/48$ , 56.25%). The prevalence of MB3s was higher in males than in females. A single molar was identified with a fourth MB canal (MB4) (0.99%) (Fig. 2).

**Table 1** Number of canals in the mesio-buccal (MB), disto-buccal (DB) and palatal (P) roots

Root	Canals	Male (n = 50)	Female (n = 51)	Total (n = 101)	Left (n = 48)	Right (n = 53)	Total (n = 101)
MB	one	20 (40%)	20 (39.22%)	40 (39.60%)	21 (43.75%)	19 (35.85%)	40 (39.60%)
	two	25 (50%)	30 (58.82%)	55 (54.46%)	24 (50%)	31 (58.49%)	55 (54.46%)
	three	4 (8%)	1 (1.96%)	5 (4.95%)	2 (4.17%)	3 (5.66%)	5 (4.95%)
	four	1 (2%)	-	1 (0.99%)	1 (2.08%)	-	1 (0.99%)
	total	50 (100%)	51 (100%)	101 (100%)	48 (100%)	53 (100%)	101 (100%)
DB	one	48 (96%)	50 (98.04%)	98 (97.03%)	47 (97.92%)	51 (96.23%)	98 (97.03%)
	two	1 (2%)	1 (1.96%)	2 (1.98%)	1 (2.08%)	1 (1.89%)	2 (1.98%)
	three	1 (2%)	-	1 (0.99%)	-	1 (1.89%)	1 (0.99%)
	four	-	-	-	-	-	-
	total	50 (100%)	51 (100%)	101 (100%)	48 (100%)	53 (100%)	101 (100%)
P	one	49 (98%)	50 (98.04%)	99 (98.02%)	47 (97.92%)	52 (98.11%)	99 (98.02%)
	two	1 (2%)	1 (1.96%)	2 (1.98%)	1 (2.08%)	1 (1.89%)	2 (1.98%)
	three	-	-	-	-	-	-
	four	-	-	-	-	-	-
	total	50 (100%)	51 (100%)	101 (100%)	48 (100%)	53 (100%)	101 (100%)

### Effect of arch sides, sex, and age

No statistically significant relationship was found between the number of canals and sides ( $P = 0.731$ ), sex ( $P = 0.183$ ) or age ( $P = 0.562$ ). In individual roots, no statistically significant relationship was found for the number of canals and sides ( $P = 0.583$ ) and sex ( $P = 0.355$ ) for the MB or other roots. No association was found between age and the total number of canals in individual roots (MB:  $P = 0.152$ ; DB:  $P = 0.192$ ; P:  $P = 0.683$ ) or the presence of the MB2 ( $P = 0.562$ ), the MB3 ( $P = 0.304$ ) or the MB4 ( $P = 0.370$ ).

### Discussion

According to the literature, the consensus is that the maxillary first molar contains either three or four canals in most individuals [Versiani MA et al. The root canal dentition in permanent dentition. Online edn. 89-240, Cham: Springer, 2019]. An early investigation by Frank Vertucci [17], considered by many as one of the pioneers of root and root canal morphological studies, established an average prevalence range of between 30% and 40% for maxillary first molars with three canals and between 60% to 70% for four canals. There are also cases where anomalies are present outside these ranges [18].

To assist practicing clinicians, various authors have attempted to provide guidelines on the common morphology that can be expected using as much diagnostic information as possible from investigations of different populations using different techniques [1,5,19]. Some of these techniques included traditional 2D radiography, [20] clearing and staining, [19] cone-beam computed tomography (CBCT) [5] and micro-CT [6]. Enhanced awareness of root canal morphology may provide practicing clinicians with a certain degree of predictability, reduce the risk of missed canals, and increase long-term tooth survival [1]. To the authors' knowledge, micro-CT studies of root canals and their variations in maxillary first molars have been generally limited, and not available at all for Black South Africans, who are the majority of the population in South Africa (79%) [Statistics South Africa. Formal Census 2011].

As micro-CT studies of the number of root canals and their variants in populations have been less common than those employing other modalities (for example CBCT), comparisons of findings with other non-micro-CT studies are included. However, when interpreting these comparisons, it is crucial to consider not only distinctions between populations but also variations in methodology. Micro-CT is often considered the gold standard for descriptions of dental morphology, [8] whereas CBCT has limited ability to identify fine root canal anatomy, and fine canals may remain undiscovered [13]. Clearing and staining techniques have been shown to have certain limitations, for example the flow of dyes into flat or irregular root canal spaces [13]. In the present investigation, the prevalence of three canals was 39.6%, while the prevalence of four canals (50.49%) was lower than that reported by Vertucci employing a clearing and staining technique in a population from the US. On the other hand, a CBCT study conducted on a Chinese population reported that four canals were found in 50.40% of

teeth [21], similar to the prevalence in the present study. The present study revealed variations of four canals between females (56.86%) and males (44%), although the findings were statistically insignificant ( $P = 0.183$ ). A prevalence of 6.93% was noted for five-canal teeth and six canals were found in 2.97% of teeth. These prevalences are higher than those reported for both five (1.8%) and six canals (0.3%) using CBCT [21,22]. Considering the relationship between age and root canals, age showed no statistically significant association with the total number of canals, canals in individual roots, or the presence of MB2, MB3 or MB4 canals.

Some case reports have described the treatment of eight canals in the maxillary first molar [18]. In the present study, however, no first molars displayed anomalies (including one, two, or more than six canals). However, a single tooth ( $n = 1/101$ ) with an uncommon C-shaped canal configuration was identified. A similar type of C-shaped configuration was described in a case report on a maxillary second molar [23]. Teeth with C-shaped root canals can have unusual root and canal anatomy that can be very complex [16]. C-shaped canal morphology is not common but can be present in maxillary first molars [24]. Using a clearing and staining technique on more than 300 teeth, this anomaly was reported to be present in 0.3% of Chinese individuals [25] residing in Taiwan, which was lower than the prevalence in this study (0.99%). The variations between this Chinese study and the present one could have been due to differences in methodology as well as sample size.

In individual roots, two canals (54.45%) were more common in the MB root of Black South Africans. A similar finding was reported in China (52.2%) [21], where more MB roots on the left side contained multiple canals than on the right (54.13% and 49.8% respectively), although the difference was not statistically significant. In contrast, the present study found more teeth with multiple canals on the right side than on the left, but the findings were also statistically non-significant ( $P = 0.731$ ). In this sample the prevalence of single-canal MB roots was 39.6%, and a similar prevalence was reported in Saudi Arabia (39.4%) [26]. The prevalence of three (4.95%) and four (0.99%) canals in the present investigation was higher than the expected prevalence of 0.1% for three and 0.4% for four canals reported elsewhere [Versiani MA et al. The root canal dentition in permanent dentition. Online edn. 89-240, Cham: Springer, 2019].

The MB2 canal in particular has been thoroughly investigated in different populations [5,14]. Although most authors used CBCT, reports of micro-CT are also available. For example, in a Japanese [27] population, a prevalence of 60.5% was reported, which was similar to that in Black South Africans (60.39%). It is interesting to note that a prevalence of 65.4% was reported in individuals of African descent resident in the US [28]. In other South African CBCT studies of other self-identified population groups, prevalences of 60.5% [Irhaime AA. Evaluation of the root and canal morphology of permanent maxillary first molars using cone-beam computed tomography in a sample of patients treated at the Wits Oral Health Centre. Dissertation, University of Witwatersrand, 2016], 89.5% [14] and 95.6% [5] were reported. Differences between populations and investigative modalities could explain this variation.

A MB3 canal, located between the MB1 and MB2 canals, can also be present in some individuals [4]. The global prevalence of this canal has been estimated to range between 0.2% and 0.8% where authors used different modalities for their determinations [29]. A literature search revealed only two geographically distant studies (from Africa, Egypt and Uganda) for comparison. Using a clearing and staining technique, the Ugandan authors did not specify the number of canals, but it seems that no teeth had more than two canals in the MB root [19]. The present study demonstrated a prevalence of 4.95%. It can be speculated that more variations might exist between African populations, but more studies will be required to confirm this.

The presence of four or more canals in the MB root is rare, and the prevalence has been estimated to be roughly 0.4% using CBCT [Versiani MA et al. The root canal dentition in permanent dentition. Online edn. 89-240, Cham: Springer, 2019]. In the present study the prevalence was 0.99%, which is slightly higher than the estimated range. The slightly higher prevalence of the MB4 canal could be attributed to the use of micro-CT, which increases the possibility of detecting additional canals, especially where the size and diameter are small [8]. Clinicians should note that population differences could also play a role, especially in a South African population [3,5,14].

The maxillary first molars included in this sample contained a single canal in 97.03% ( $n = 98/101$ ) of DB roots. Previous authors have reported a global prevalence of 1.4% for additional canals in the DB root, considering different modalities [Versiani MA et al. The root canal dentition in permanent dentition. Online edn. 89-240, Cham: Springer, 2019]. In 1.98% of DB roots in the present sample, an additional canal was found and one tooth had a third additional canal ( $n = 1/101$ , 0.99%), giving a total prevalence of 2.97% ( $n = 3/101$ ). Studies of additional distal canals using micro-CT have been limited, and none involving African populations have been identified. However, in the Ugandan study mentioned earlier, Rwenyonyi et al. [19], reported a prevalence of 2.3%. The slightly higher prevalence noted in the present study could have been due to differences between investigative modalities.

The global prevalence of additional canals in the P root has been estimated to be 0.7% [Versiani MA et al. The root canal dentition in permanent dentition. Online edn. 89-240, Cham: Springer, 2019]. A higher prevalence of 1.98% ( $n = 2/101$ ) was determined in the present study and most P roots contained a single canal ( $n = 99/101$ , 98%). In a Ugandan population, no additional P canals were found, but additional P canals were identified in 1% of maxillary first molars of Nigerians [30]. In both the Nigerian study and the present one, the prevalence was higher than the estimated global prevalence.

Modalities, sample sizes and methodology differ between studies and may influence the reported prevalence of root canal and root variations across populations. Anatomical variations can also be attributed to genetics, population groups, and geographical location amongst other factors [3-6,19]. The authors of this study chose micro-CT, which can discern fine detail that makes it easier to identify additional canals [13]. Population differences could also be a contributing factor. It seems that the prevalence of additional canals in DB and P roots in some African populations (South Africa, Nigeria and Uganda) is higher than in other populations, considering the global prevalence. Despite this, variation in root canal morphology should be anticipated as it plays an important role in root canal treatment. Modern populations can include individuals from diverse backgrounds [2011 Census Analysis: Ethnicity and Religion of the Non-UK Born Population in England and Wales. Office of National Statistics, 2011]

One limitation of this study was the use of dried human skulls. In a minority of the scans examined, dentinal cracks were observed. The masking or multiple-slice editing technique [13] was able to reduce minor dentinal cracks to a degree sufficient for observation purposes without compromising any detail. Other limitations included the relatively small sample size ( $n = 101$ ) and the fact that only one population group was investigated. Also, no comparison was made with other population groups within South Africa.

## Abbreviations

2D: two-dimensional; 3D: three-dimensional; CBCT: cone-beam computed tomography; CEJ: cemento-enamel junction; DB: disto-buccal; HOCR: Human Osteological Research Collection; MB: mesio-buccal;

MB2: second mesio-buccal canal; MB3: third mesio-buccal canal; MB4: fourth mesio-buccal canal; micro-CT: micro-computed tomography; P: palatal; PBC: Pretoria Bone Collection; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

## Ethical Statements

Ethical approval was granted by the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (Protocol number: 298/2020), before the study commenced.

## Conflicts of Interest

The authors declare that there are no conflicts of interest relating to this study.

## Funding

This publication was funded/co-funded by the European Union. Its contents are the sole responsibility of Jonker, CH and authors, and do not necessarily reflect the views of the European Union. Dr. Charlotte Theye received funding from the Sefako Makgatho Health Sciences University through the Bakeng se Afrika project funded by Erasmus plus, EU funding: 597924-EPP-1-2018-1-ZA-EPPKA2-CBHE-JP (2018-3229).

## Author Contributions

CHJ: conceptualization, investigation, methodology and writing; ENL: writing: review and editing; PVDV: writing: review and editing, visualization; DZ: data analysis, writing: review and editing; ACO: conceptualization, writing: review and editing. All authors read and approved the final version of the manuscript.

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## Acknowledgments

The authors acknowledge the statistical support of Dr. Charlotte Theye in preparation of this paper. The authors also extend their gratitude to Dr. Guy Lambourn for participating in the inter-reliability test.

## Data Availability Statements

Data generated during the current study are available from the corresponding author on reasonable request.

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