

## Original Article

**A micro-computed tomographic evaluation of maxillary first molar accessory root canal morphology in a Black South African subpopulation**Casper H. Jonker\*<sup>1)</sup>, Peet J. van der Vyver<sup>2)</sup>, Guy Lambourn<sup>1)</sup>, and Anna C. Oettlé<sup>3)</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 Odontology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa<sup>3)</sup>Anatomy and Histology Department, School of Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa

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**Abstract****Purpose:** The aim of this study was to investigate the accessory root canal morphology of maxillary first molars in a Black South African subpopulation.**Methods:** Micro-computed tomography was used to investigate 101 maxillary first molars (from 50 male and 51 female teeth, right 53 teeth, left 48 teeth). The prevalence of chamber canals, and the number, type and location (root third) of accessory canals were recorded. The relationships between arch side, sex and age were examined using chi-squared tests of association. Intra- and inter-observer reliability were assessed using Cohen's kappa test.**Results:** Intra- and inter-rater agreement was 96.9% and 98.1%, respectively. Variations in accessory root canal anatomy according to side, sex and age were evident. Chamber canals were identified in 10.9% of teeth. Accessory canals were found mainly in the apical third of most teeth in the sample, and distributed predominantly in the mesio-buccal root. Apical deltas were most prevalent in the mesio-buccal root, and their frequency decreased in the palatal and then finally the disto-buccal root.**Conclusion:** Accessory root canals were common in this population, and showed a diverse range of anatomy. The present findings will be of assistance to clinicians during endodontic treatment and will also be valuable for educational purposes.

Keywords: accessory canal, apical delta, chamber canal, micro-CT

**Introduction**

Once the pulpal space of a tooth becomes irreversibly inflamed, endodontic intervention is required, involving the removal of inflamed or infected pulpal contents in a series of mechanical and chemical steps followed by a three-dimensional (3D) seal of the prepared spaces [1]. Although for first and second molars the success rate of root canal treatment can be more than 90%, treatment can still fail despite a clinician's best efforts [2]. To maximize the success rate, it is important for all areas of the root canal system to be treated [1]. One reason for treatment failure is complexity of the root canal anatomy, and in this context it is important to note that the morphology of the pulpal network differs between populations and among individuals [Versiani MA, The root canal dentition in permanent dentition. Online edn: 89-240, Cham: Springer, 2019]. Geographic origins, external factors, sex and genetics may all contribute to variations among individuals [3,4].

The root canal system may include an intricate network of accessory canals (ACs), which can be situated in the coronal, midroot, or apical third of the root and may exhibit characteristics described as patent, blind-ended, or looped [5]. Additionally, chamber canals (CCs) may exist, and main root canals might culminate in apical deltas featuring multiple portals of exit or ramifications [1,5]. Understanding the anatomy of accessory root canals is

crucial for the success of treatment, as they can be potential pathways for infection and harbor infected organic material [1,6].

In the past, fine details and complexities within the root canal system have often remained hidden, as traditional radiographs only provide a two-dimensional view of a tooth [7]. More recently, micro-computed tomography (micro-CT) has emerged as a non-invasive and accurate modality for visualizing the internal morphology of teeth in three dimensions. Since the first report documenting the use of micro-CT for clarifying the internal and external root and canal anatomy of a maxillary first molar by Nielsen et al. in 1995 [8], this modality is has gained acceptance as the most suitable means of investigating fine root and canal morphology [5-7,9].

The aim of the present study was to utilize micro-CT to provide basic data on accessory root canal anatomy and its variants in maxillary first molars of a Black South African subpopulation.

**Materials and Methods**

Prior to the study, ethical approval was obtained from the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (Protocol number: 298/2020). The study followed a quantitative, descriptive, cross-sectional and observational design. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were also considered [10] (Fig. 1).

**Origin of scans**

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) were sourced for samples of human skulls with known age, sex and population affinity [11]. Family members of the deceased individuals had granted permission for research, and these bodies formed part of the whole-body donation programme. Their use for research purposes was approved by the director general for third party donations. All the bodies were 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)]

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) micro-focus X-ray computed tomography scanner with the following settings: 100 kV voltage, 100 mA current and 2.0 s exposure time per projection. The spot size of the X-ray unit ranged between 0.001 and 0.003 mm (1-3  $\mu$ m) and the translation table of the unit had a rotation accuracy to 1/1,000th of a degree and a pixel size of 200  $\mu$ m  $\times$  200  $\mu$ m. The Perkin Elmer detector of the unit had a 400 mm  $\times$  400 mm field of view capacity, and a field of view of approximately 200 mm  $\times$  200 mm was used to scan each maxilla [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]. Two-dimensional projection images were reconstructed after completion of the scanning process with the Nikon CT Pro version 4.4.3 software (Nikon Metrology) into 3D volumes. Isotropic voxel size/scan resolution ranged between 40  $\mu$ m and 62  $\mu$ m. On completion, the final 3D volumes were imported into

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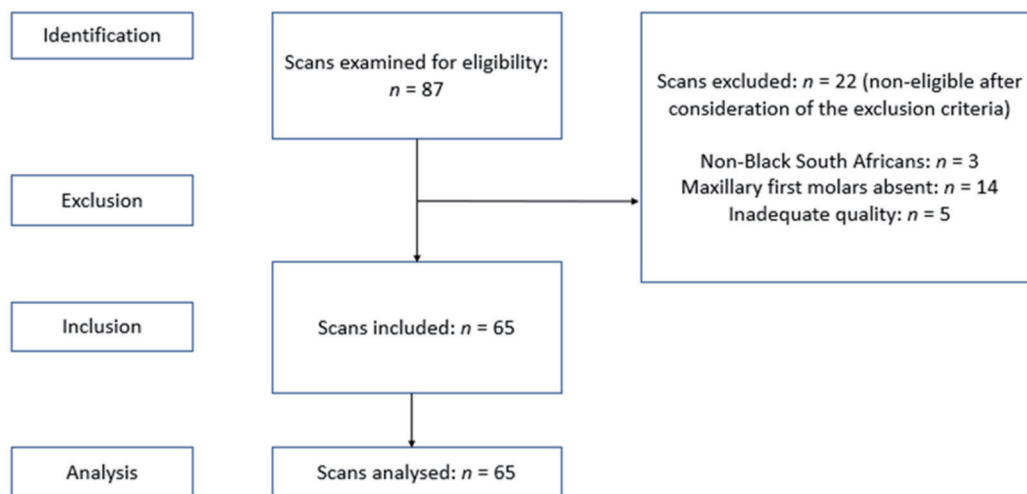


Fig. 1 STROBE flow diagram to illustrate the process followed to determine eligibility of available scans

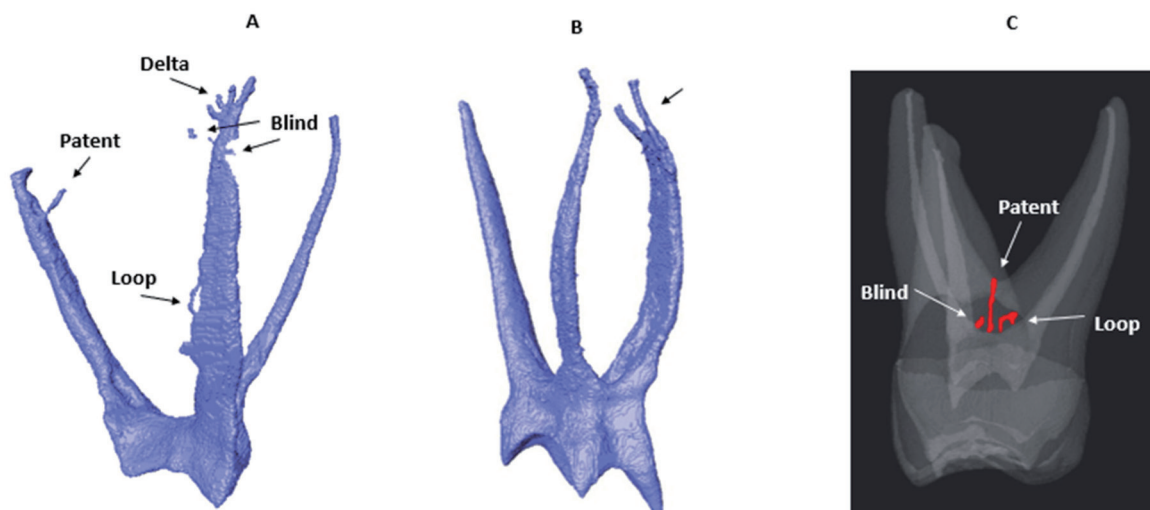


Fig. 2 (A) Micro-CT illustrations of ACs (patent, looped and blind-ended) and apical deltas based on the descriptions and illustrations of Ahmed et al. [5,14], (B) Root canal bifurcations based on the guidelines and illustrations described by Xu et al. [16-18] (arrow), (C) Illustrative depictions of CCs (red) based on the descriptions by Ahmed et al. [5,14]

Avizo 2019 (Thermo Fisher Scientific, Waltham, MA, USA), a 3D visualization software package, 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].

#### 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 extracted virtually by cropping and segmenting each scan. Landmarks were placed on each tooth in the maxillary arch using the cemento-enamel junction (CEJ) as a guide. For molars, one landmark was placed at the highest occlusal point of each root on both the buccal and palatal surfaces along the CEJ (three or four landmarks in total). The CEJ is commonly used as a readily identifiable site of reference [12].

A best-fit plane was automatically computed at the level of the CEJ from these landmarks, which was used as a reference to re-align the micro-CT image stacks. The possibility of introducing oblique sections and possible bias was reduced by repeating these steps for each scan.

To allow virtual extraction and 3D observation, a watershed method (region-based semi-automatic segmentation) was carried out [13]. Segmentation is a process by which each tooth and its inner components (enamel, dentin and pulp) are virtually isolated from each other. Different colors were used to enhance the differentiation between components. A masking or multiple-slice editing procedure [14] was carried out to carefully edit

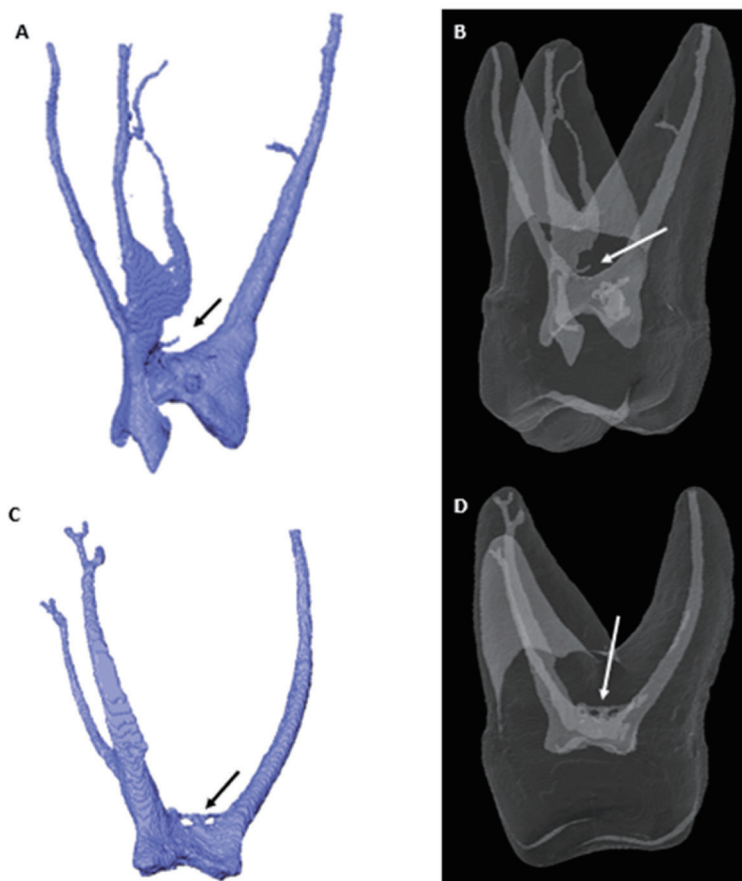
and correct slices where teeth had minor dentinal cracks in communication with the pulpal space or exterior spaces beyond the roots.

#### Scan analysis

Observation of scans was performed using a pattern similar to that described by Jonker et al. [15]. Data on accessory root canal anatomy was first collected by the main researcher, who was proficient with the use of the Avizo software and experienced in the field of endodontics. Unique codes were allocated to each scan, and data capturing was completed anonymously without prior knowledge of sex, arch side or age. The pulps from each scan were isolated, magnified and rotated using settings and parameters within Avizo 2019. By adjusting the brightness, contrast and sharpness parameters within the software, optimal imaging was achieved. The same parameters were used continuously for each scan.

Data capture included the number and location of ACs by root thirds (coronal, midroot or apical) and AC types (loops, patent and blind-ended). The guidelines of Ahmed et al. [5,14] were followed during observation, as those authors had provided clear descriptors to allow more comparability between studies. Guidelines and illustrations provided by Xu et al. [16-18] and Ahmed et al. [14] were also considered (Fig. 2A-C). For this study, root canal bifurcations were identified as root canal branches where the main root canal divided into two similar-sized branches separated by an acute angle and each had separate exits on the apical root surface (Fig. 2B).

After data capture, a specialist in prosthodontics with endodontic experience participated in inter-observer reliability testing. Prior to the



**Fig. 3** Micro-CT images of types of CCs observed in this study, (A) Blind-ended CCs (black arrow), (B) Blind-ended CCs illustrated via the pulp space view (white arrow), (C) Looped CCs (black arrow), (D) Looped-type CCs illustrated via the pulp space view (white arrow)

test, researchers were calibrated by observing and discussing the guidelines described by Ahmed et al. [5,14] and Xu et al. [16-18] followed by observation of micro-CT images of accessory root canal anatomy in two randomly chosen scans unrelated to the main reliability test. During the test, researchers recorded their results independently and the results were compared afterwards. In the event of agreement, results were accepted and any discrepancies were discussed until a consensus was reached. Intra-rater reliability was also determined by repeating each observation on the same selection of scans by the main researcher within one week [19].

#### Inclusion criteria

High-resolution scans (without blurring or double imaging) of teeth from Black South Africans with intact roots and fully developed apices, where the pulp space could be accurately isolated, were included.

#### Exclusion criteria

Teeth with immature apices (incomplete root formation), root fractures, coronal or radicular resorption, existing root canal treatments, extensive decay where any root canals were obscured, metal restorations, scans of suboptimal quality and other self-identified population groups were excluded.

#### Sample size

All available scans ( $n = 87$ ) were considered (convenience sample) (Fig. 1). A total of 101 maxillary first molars from 65 scans were identified after consideration of the inclusion and exclusion criteria. The final sample consisted of teeth from males and females of different ages from the left and right sides of the arch.

#### Statistical analysis

RStudio 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 to perform the statistical analysis. The relationship between arch side, sex and age and accessory root canal anatomy was analyzed using chi-squared test of association ( $P < 0.05$ ). To determine intra- and inter-observer reliability, Cohen's kappa coefficient test was used. The percentage of agreement was determined by evaluating randomly selected scans for approximately 20% of the sample size ( $n = 20/101$ ) [15].

## Results

#### Sample characteristics and examiner agreement

The sample included 53 teeth on the right side ( $n = 53/101$ , 52.5%) and 48 on the left ( $n = 48/101$ , 47.5%); 50 teeth were from males ( $n = 50/101$ , 49.5%) and 51 ( $n = 51/101$ , 50.5%) were from females. The age range was 20-70 years, with a mean age of 41.84 years. Intra- and inter-rater reliability was 96.9% and 98.1% (high agreement between raters) with kappa values of 0.94 and 0.96, respectively.

#### Chamber canals

CCs were identified in 10.9% of the sample ( $n = 11/101$ ). In two molars, looped CCs were found ( $n = 2/101$ , 2.0%) (Fig. 3C, D) but no patent CCs were identified. The most common type of CC was blind-ended ( $n = 9/101$ , 8.9%) (Fig. 3A, B), and a higher prevalence was found in males ( $n = 6/101$ , 5.9%) than in females ( $n = 3/101$ , 3.0%).

#### Total number of accessory canals

A total of 281 ACs (excluding deltas) were identified. The mean number of ACs per tooth was 2.78 (SD: 1.85; median: 3.00; range: 0.00-6.00). The findings for each root are summarized as follows:

#### Accessory canals in the mesio-buccal root

Findings specific to the mesio-buccal (MB) root are summarized in Table 1. A total of 141 individual ACs (excluding apical deltas) were counted

**Table 1** Number and frequency of accessory canals in the MB root including regions, types, deltas and number of accessory canals

		Male (n = 50)	Female (n = 51)	Left (n = 48)	Right (n = 53)	Total (n = 101)
Teeth with ACs	total:	44 (88.0%)	42 (82.4%)	40 (83.3%)	46 (86.8%)	86 (85.2%)
ACs by region	coronal	7 (14.0%)	9 (17.7%)	5 (10.4%)	11 (20.8%)	16 (15.8%)
	midroot	10 (20.0%)	16 (31.4%)	12 (25.0%)	14 (26.4%)	26 (25.7%)
	apical	42 (84.0%)	34 (66.7%)	36 (75.0%)	40 (75.5%)	76 (75.3%)
ACs type or delta	loops	4 (8.0%)	7 (13.7%)	3 (6.3%)	8 (15.1%)	11 (10.9%)
	blind	13 (26.0%)	18 (35.3%)	15 (31.3%)	16 (30.2%)	31 (30.9%)
	patent	31 (62.0%)	24 (47.1%)	26 (54.2%)	29 (54.7%)	55 (54.5%)
	delta	12 (24.0%)	10 (19.6%)	9 (18.8%)	13 (24.5%)	22 (21.8%)
Number of ACs (excluding apical deltas)	0	14 (28.0%)	15 (29.4%)	15 (31.3%)	14 (26.4%)	29 (28.7%)
	1	16 (32.0%)	15 (29.4%)	14 (29.2%)	17 (32.1%)	31 (30.7%)
	2	14 (28.0%)	11 (21.6%)	13 (27.1%)	12 (22.6%)	25 (24.7%)
	3	3 (6.0%)	5 (9.8%)	3 (6.3%)	5 (9.4%)	8 (7.9%)
	4	2 (4.0%)	3 (5.9%)	2 (4.2%)	3 (5.7%)	5 (6.0%)
	5	1 (2.0%)	1 (2.0%)	1 (2.1%)	1 (1.9%)	2 (2.0%)
	6	-	1 (2.0%)	-	1 (1.9%)	1 (1.0%)
Total ACs		66 (100%)	75 (100%)	62 (100%)	79 (100%)	141 (100%)

**Table 2** Number and frequency of accessory canals for the DB root including regions, types, deltas and number of accessory canals

		Male (n = 50)	Female (n = 51)	Left (n = 48)	Right (n = 53)	Total (n = 101)
Teeth with ACs	total:	38 (76.0%)	29 (56.9%)	30 (62.5%)	37 (69.8%)	67 (66.3%)
ACs by region	coronal	1 (2.0%)	2 (3.9%)	1 (2.1%)	2 (3.8%)	3 (3.0%)
	midroot	5 (10.0%)	1 (2.0%)	3 (6.3%)	3 (5.7%)	6 (5.9%)
	apical	36 (72.0%)	29 (56.9%)	28 (58.3%)	37 (69.8%)	65 (64.4%)
ACs type or delta	loops	3 (6.0%)	4 (7.8%)	1 (2.1%)	6 (11.3%)	7 (6.9%)
	blind	8 (16.0%)	5 (9.8%)	7 (14.6%)	6 (11.3%)	13 (12.9%)
	patent	31 (62.0%)	20 (39.2%)	19 (39.6%)	32 (60.4%)	51 (50.5%)
	delta	5 (10.0%)	4 (7.8%)	5 (10.4%)	4 (7.6%)	9 (8.9%)
Number of ACs (excluding apical deltas)	0	15 (30.0%)	26 (51.0%)	23 (47.9%)	18 (34.0%)	41 (40.6%)
	1	19 (38.0%)	18 (35.3%)	15 (31.3%)	22 (41.5%)	37 (36.6%)
	2	13 (26.0%)	5 (9.8%)	9 (18.8%)	9 (17.0%)	18 (17.8%)
	3	2 (4.0%)	2 (2.0%)	1 (2.1%)	3 (5.7%)	4 (4.0%)
	4	1 (2.0%)	-	-	1 (1.9%)	1 (1.0%)
	5	-	-	-	-	-
	6	-	-	-	-	-
Total ACs		55 (100%)	34 (100%)	36 (100%)	53 (100%)	89 (100%)

(mean average: 1.40; SD: 1.31; median: 1; range: 0.00-6.00), located in 85.2% of the MB roots of which the majority were present in the apical third with a slight preponderance for the right side. All three types of ACs were noted in the total sample, with patent ACs occurring more commonly in males. In contrast, blind-ended and looped ACs were more common in females. Apical deltas were found in 21.8% of roots and more commonly in males.

#### Accessory canals in the disto-buccal root

Findings specific to the disto-buccal (DB) root are summarized in Table 2. A total of 89 ACs (excluding apical deltas) were counted (mean average: 0.9; SD: 1.00; median: 1.00; range: 0.00-4.00), located in 66.3% of the DB roots of which the majority were present in the apical third with a slight preponderance for the right side. All three types of ACs were noted in the total sample, with blind-ended and patent ACs occurring more commonly in males. There was a similar occurrence of loops and deltas in both sexes.

#### Accessory canals in the palatal root

Findings specific to the palatal (P) root are summarized in Table 3. A total of 51 ACs (excluding apical deltas) were counted (mean average: 0.50; SD: 0.69; median: 0.00; range: 0.00-3.00), located in 51.5% of the P roots of which the majority were present in the apical third with a slight preponderance for the left side. All three types of ACs were noted in the total sample, the most commonly occurring being the patent type.

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

For the MB root, the relationship between root third and ACs (including apical deltas) was not significant for arch side ( $P = 0.495$ ) or sex ( $P = 0.290$ ). Similarly, no statistically significant relationship was found for the MB root between AC type (sexes:  $P = 0.442$ ; sides:  $P = 0.616$ ) and number

(sexes:  $P = 0.909$ ; sides:  $P = 0.935$ ). For DB and P roots, no statistically significant relationship was noted between ACs and the root third (DB and P sexes:  $P = 0.325$ ,  $P = 0.824$ ; DB and P sides:  $P = 0.232$ ,  $P = 0.294$ ). There was also no significant relationship between ACs type (DB and P sexes:  $P = 0.862$ ,  $P = 0.862$ ; DB and P sides:  $P = 0.258$ ,  $P = 0.862$ ) or number (DB and P sexes:  $P = 0.111$ ,  $P = 0.292$ ; DB and P sides:  $P = 0.449$ ,  $P = 0.687$ ). Finally, when considering age, no statistically significant relationship was noted for MB and DB roots ( $P = 0.624$ ) but a significant higher prevalence of ACs was noted in the P root ( $P = 0.037$ ).

## Discussion

Knowledge of root canal morphology is essential for better clinical outcomes and can increase the long-term survival of teeth subjected to root canal treatment [1]. Comprehensive morphological information on diverse populations can assist clinicians in achieving an optimal endodontic result [1,20]. Past and current investigative techniques have included the use of traditional two-dimensional (2D) radiographs [21], in vitro macroscopic and clinical investigations [22], clearing and staining [23], cone-beam computed tomography (CBCT) imaging [20] and micro-CT [6] amongst others. No previous studies have reported on the accessory root canal morphology of Black South Africans, and a need for such data has been identified as these individuals are by far the largest population group in South Africa (79%) [Statistics South Africa. Formal Census 2011]. A review of the literature failed to find any other comprehensive study of accessory canals of first maxillary molars using micro-CT for any population group.

Accessory root canal anatomy is difficult or even impossible to visualize using traditional radiography [8,14]. The presence of ACs and apical deltas cannot be ignored as they are often found in human teeth and create

**Table 3** Number and frequency of accessory canals for the P root including regions, types, deltas and number of accessory canals

		Male (n = 50)	Female (n = 51)	Left (n = 48)	Right (n = 53)	Total (n = 101)
Teeth with ACs	total:	29 (58.0%)	23 (45.1%)	27 (56.3%)	25 (47.2%)	52 (51.5%)
ACs by region	coronal	-	-	-	-	-
	midroot	2 (4.0%)	3 (5.9%)	1 (2.1%)	4 (7.6%)	5 (5.0%)
	apical	27 (54.0%)	21 (41.2%)	26 (54.2%)	22 (41.5%)	48 (47.5%)
ACs type or delta	loops	1 (2.0%)	1 (2.0%)	1 (2.1%)	1 (1.9%)	2 (2.0%)
	blind	6 (12.0%)	4 (7.8%)	4 (8.3%)	6 (11.3%)	10 (9.9%)
	patent	16 (32.0%)	14 (27.5%)	16 (33.3%)	14 (26.4%)	30 (29.7%)
	delta	6 (12.0%)	8 (15.7%)	6 (12.1%)	8 (15.1%)	14 (13.9%)
Number of ACs (excluding apical deltas)	0	27 (54.0%)	33 (64.7%)	27 (56.3%)	33 (62.3%)	60 (59.4%)
	1	17 (34.0%)	15 (29.4%)	17 (35.4%)	15 (28.3%)	32 (31.7%)
	2	5 (10.0%)	3 (5.9%)	4 (8.3%)	4 (7.6%)	8 (7.9%)
	3	1 (2.0%)	-	-	1 (1.9%)	1 (1.0%)
	4	-	-	-	-	-
	5	-	-	-	-	-
	6	-	-	-	-	-
Total ACs		30 (100%)	21 (100%)	25 (100%)	26 (100%)	51 (100%)

communication portals between the pulp and the periodontal ligament space. They contain blood vessels, which are encapsulated during the Hertwig root sheet (HERS) developmental stage [Versiani MA. The root canal dentition in permanent dentition. Online edn: 31-46, Cham: Springer, 2019]. ACs are also challenging to reach and disinfect with currently available instruments and disinfection agents.

A specific type of AC, namely CCs, may be a possible route of communication between the periodontal space and the pulp in the furcation region [24]. In the present study a prevalence of 10.9% was noted, which included two looped-type CCs. In a micro-CT study of German and Egyptian extracted first molars, a prevalence of 2.8% was reported [24]. In the same study, a higher prevalence of blind-ended CCs was found, similar to the present study. In contrast, no patent CCs were identified in the present study whereas in the German and Egyptian study a prevalence of 2.2% was reported for patent CCs. These differences between the studies could be attributable to a variety of factors including geographic origin, external factors, sex, sample size and genetics [4,25-27].

The present study revealed that the MB root had the highest number of ACs (85.2%), followed by the DB and P roots (66.3% and 51.5% respectively). Similar findings were reported in a Japanese study, where the investigators determined that the MB root contained the most diverse root canal morphology of all three roots [25]. The current investigation found that ACs occurred most commonly in the apical third of the root, which is in agreement with other studies. In the present sample, 141 ACs (50.2%) were found in the MB root, ranging between 0 and 6 per tooth. In a Burmese (Myanmar) population, 56.7% of all ACs were found to be in the MB root [27], and on average, each tooth contained approximately 2.22 ACs, being higher than in the present study with a mean average of 1.4 ACs. An interesting finding in the current investigation was that a higher prevalence of ACs was noted for the right side (79 on the right compared to 62 on the left). However, there was no significant relationship between ACs and age in the MB root.

In the present study, more apical deltas were identified in the MB root than in the other roots (a prevalence of 21.8%), which is similar to findings in a Chinese population [28]. Unfortunately, in that Chinese study, mainly maxillary molars were studied and no distinction was made between first and second molars. A much lower prevalence of 6.0% was reported in a Brazilian micro-CT study [29]. However, the authors used descriptors and terminology different from those in the present study, which adopted the Ahmed et al. terminology for apical deltas. The use of different descriptions and classification systems complicates comparisons. Sert and Bayirli [3] also observed that more apical deltas were present in molars from females than in those from males, whereas in the present study teeth from males were more likely to contain deltas in the MB root. It is also interesting to note that in the present study the right side contained more apical deltas than the left (18.8% on the left and 24.5% on the right).

When considering the DB root, a study of a Burmese population by Kyaw Moe et al. [27] found 23.8% of total ACs in the DB root, being lower than the 31.7% in the present investigation. It can be speculated that geographic location or perhaps genetics could explain these differences

between findings [5,16,24]. A right-sided predominance was also noted in the present study, but this was not statistically significant. The relationship between age and ACs was also not significant for the DB root in the present study. The overall prevalence of apical deltas in the DB root was 8.9%, being higher in males (10.0%) than in females (7.8%). A different finding was reported in a Turkish study in which greater numbers were seen in females than in males [3]. Also, more apical deltas were identified in the DB root in the present study than in a Chinese micro-CT study, where a prevalence of 1.7% was noted. However, as stated before, the Chinese sample contained both first and second molars, so an accurate comparison was not possible [28].

In the present study, ACs were less frequent in the P root (prevalence of 18.2%), which is in accordance with the findings of other studies [30]. In contrast, an Irish study found that the P root had the highest prevalence of ACs compared to the DB or MB [31]. The most common location for ACs in the present investigation was the apical third of the root, which is similar to findings in other populations [4,17,30,31]. The present study also found, on average, at least one AC present in 51.5% of P roots. Using micro-CT, a lower prevalence (40.4%) was reported by Divine and colleagues [32] in a population from the USA, but no patient demographics were reported. In the present study, the average prevalence of apical deltas in P roots was 13.9%, whereas in a Chinese population the prevalence was less than 1.1% [28]. In the present study, a slightly higher prevalence for apical deltas was noted in females (15.7%) than in males (12.0%), which is similar to findings in a Turkish population [3]. Interestingly in the current investigation, the effect of age on ACs in the P root was found to be statistically significant ( $P = 0.037$ ), with a higher incidence in older individuals. It can be speculated that increased dentin deposition occurs between the narrowest point of a canal and the root canal wall as age increases, resulting in a higher prevalence of accessory root canal anatomy. This speculation would support an early finding by Hess and Zürcher in 1925 [Hess W, Zürcher E. The anatomy of the root canals of the permanent dentition. Part 1. 3-49, New York: William Wood and Company, 1925].

It is important to note that even though modalities and methodologies have differed between studies and could have contributed to variations between populations, true anatomical variations are important for clinicians to consider. The findings of the present investigation may contribute to successful treatment outcomes, as modern populations also have diverse backgrounds [Ethnicity facts and figures: Population in England and Wales 2022 (Updated 21 May 2024)].

A possible limitation of this study was the use of dried human skulls, which can lead to micro-crack formation possibly confused with accessory canal anatomy. This limitation was addressed by removing dentinal cracks using masking or multiple-slice editing, as described by Ahmed et al. [14]. To facilitate comparisons between sides, micro-CT scanning was done on the entire skeletonized maxillae, resulting in larger voxel sizes (between 40  $\mu\text{m}$  and 62  $\mu\text{m}$ ) for this study. In comparison, similar scans on extracted teeth have reported smaller voxel sizes ranging between <10  $\mu\text{m}$  and 30  $\mu\text{m}$  [6,7,14]. At larger voxel sizes the patency of some ACs may not have been evident. The authors acknowledge that a degree of subjectivity in the

identification of root canal anatomy could exist, for example distinction between accessory canal types or distinction between apical accessory canals and apical bifurcating canals. However, the outcome of intra- and inter-reliability testing and consensus between researchers would suggest that subjectivity was reduced to a minimum. Finally, the study included only one population group and no comparison was made to other population groups within South Africa, and the sample size ( $n = 101$ ) can also be considered a limitation.

The accessory root canal anatomy of maxillary first molars in this population was found to be diverse. The MB root had the highest prevalence of ACs and these were located predominantly in the apical third. The P root was also more likely to contain ACs with increased age. Both of these findings have direct implications for clinical treatment and outcomes, at least in the context of maxillary first molars in Black South Africans.

## Abbreviations

2D: two-dimensional; 3D: three-dimensional; AC: accessory canal; CBCT: cone-beam computed tomography; CEJ: cemento-enamel junction; CC: chamber canal; DB: disto-buccal; HOCR: Human Osteological Research Collection; MB: mesio-buccal; micro-CT: micro-computed tomography; P: palatal; PBC: Pretoria Bone Collection; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

## Ethical Statements

Prior to the study, ethical approval was obtained and granted by the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (Protocol number: 298/2020).

## Conflicts of Interest

The authors have no conflicts of interest to declare.

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## Author Contributions

CHJ: conceptualization, investigation, methodology, writing and visualization; PVDV: writing: review and editing, visualization; GL: writing: review and editing, visualization; ACO: conceptualization, writing: review and editing. All authors read the final version of the manuscript and are in agreement on the content.

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## Data Availability Statements

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

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