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Cranial fluctuating asymmetry and its relationship with non-specific physiological stress indicators in a contemporary South African cadaveric skeletal sample

M. Harripershad [*,1], C.E.G. Theye [2], A.F. Ridel [3], L. Liebenberg [4]

Department of Anatomy, Faculty of Health Sciences, University of Pretoria, Private Bag x323, Gezina, Pretoria 0031, South Africa

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ABSTRACT

Objectives: Biological anthropologists frequently explore skeletal asymmetry, together with population health and disease. Given the conflicting findings in existing literature, this study aimed to clarify whether an association exists in a South African sample.
Materials: Dry bone and cranial micro-focus X-ray Computed Tomography (micro-XCT) scans of 115 South African individuals were assessed.
Methods: Fluctuating asymmetry (FA) indices were calculated from interlandmark distances, and the frequency of four types of non-specific signs of physiological stress were documented to explore the relationship between FA and disease.
Results: Black South Africans did not exhibit a high FA index; however, they had the highest prevalence of non-specific signs of physiological stress. However, no significant correlations were detected between FA indices and pathological lesions.
Conclusion: No correlation was observed between FA and populations from different socio-economic backgrounds. However, individuals of lower socio-economic status (SES) demonstrated a greater prevalence of non-specific signs of physiological stress.
Significance: This research suggests that skeletal indicators of stress may be a suitable biological marker for assessing differences in SES among population groups, while indicating that levels of cranial FA is an inadequate biological marker.
Limitations: Possible limitations may include measurement error, and the lack of information on the life history and medical records of individuals in this sample.
Suggestions for further research: Future research should include a larger sample with more South African groups, and should evaluate the potential association among age, FA, and expression of skeletal markers of disease.

1. Introduction

The impact of genetic and environmental stressors on human health is of great interest to biological anthropologists (Lewis, 2016; Sparacello et al., 2017; Mays and Brickley, 2018; Betsinger and DeWitte, 2020). Certain markers of developmental or physiological stress can be assessed to provide a record of an individual's lived experience in their developmental environment (Weisensee and Spradley, 2018). By assessing population history and socio-economic status (SES) in relation to fluctuating asymmetry and skeletal signs of stress, anthropologists can measure its functional effects on growth and development (Hagg et al., 2017; Weisensee and Spradley, 2018).

* Correspondence to: Department of Anatomy, University of Pretoria, Private Bag x323, Arcadia, 0007, South Africa.
E-mail addresses: miksha.harripershad@gmail.com (M. Harripershad), charlotte.theye@gmail.com (C.E.G. Theye), alison.ridel@up.ac.za (A.F. Ridel), leandi.liebenberg@up.ac.za (L. Liebenberg).
[1] 0000–0002-5279–9067
[2] 0000–0002-4756–8854
[3] 0000–0002-9129–423X
[4] 0000–0003-4310–5489

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1.1. Fluctuating asymmetry, developmental stress, and social disparities

Organisms are considered perfectly symmetrical when in constant homeostasis with their internal and external environments (Graham and Özener, 2016). Homeostasis is controlled by self-regulating mechanisms intended to maintain developmental stability which may produce the "ideal" phenotype, and thus perfect symmetry (Graham and Özener, 2016). Therefore, the presence of genetic and/or environmental stress can disturb homeostasis and may result in inconsistencies in growth and development, leading to asymmetry (Graham and Özener, 2016). Three categories of asymmetry have been described in the literature: (1) fluctuating asymmetry, (2) directional asymmetry, and (3) anti-symmetry. Since it is easily quantifiable, directional asymmetry is a popular focus or research among anthropologists. On the other hand, FA tends to be overlooked, especially in contemporary human samples ((Latimer and Lowrance, 1965; Hiramoto, 1993; Cuk et al., 2001; Auerbach and Ruff, 2006; Drapeau, 2008;;Kanchan et al., 2008) Fluctuating asymmetry, the term commonly used to describe asymmetry associated with stressors, refers to the random deviation from perfect symmetry, with inequality in the size or shape of bilateral (left and right-sided) traits (Graham and Özener, 2016). Hence, improved knowledge and understanding of the role played by developmental stressors and their correlation to skeletal asymmetry enable anthropologists to better interpret human variation and understand the effects on human health.

Childhood developmental stress usually arises from congenital defects, living in poverty, nutritional defects, and/or susceptibility to disease. Children living in low SES conditions have been reported to require hospitalisation more frequently due to increased susceptibility to disease which consequently leads to more health problems throughout their lifetime (Coolidge, 2015). Literature also reports a higher degree of FA in populations with lower SES, particularly if foetal development occurred while the mother underwent physiological stress (Harris and Nweeia, 1980; Livshits et al., 1988; Gawlikowska et al., 2007; Storm, 2009). Özener (2010) observed increased levels of facial FA in low SES groups among individuals from different environmental conditions, and also noted that males had a higher FA score compared to females. FA has been hypothesised to be useful as a proxy to study developmental defects that arise during early development and may continue to affect adult health (Livshits et al., 1988).

Studies have correlated the presence of skeletal indicators of physiological stress (linked to nutritional and environmental stress) to increased levels of FA (DeLeon, 2007; Hoover, 2007;Weisensee, 2013; O'Donnell and Moes, 2021); for instance, high levels of FA have been associated with the presence of cribra orbitalia and enamel hypoplasia. In a historic Dutch sample, Hagg et al. (2017) observed that individuals with pathological lesions were skeletally more asymmetrical than those without any visible lesions. This paper recommended that future research may benefit from considering the socio-political and environmental history of a population (specifically during development), to clarify if FA can be used as an indicator of developmental stability (Hagg et al., 2017). Moreover, while FA develops from infancy into early childhood, which is consistent with the development of skeletal markers of physiological stress, Storm (2009) and Hallgrímsson (1999) reported that FA continued to increase into adulthood after the age of 20 years. Research on the correlations between FA and skeletal markers of physiological stress can thus provide information on the lived and continued experiences of individuals.

1.2. Non-specific indicators of physiological stress and social disparities

Non-specific skeletal markers of disease most often develop during childhood due to early life stress and can remain visible throughout adulthood (Anderson, 2022). Yaussy (2019) explored the potential correlation of low SES with the presence of cribra orbitalia, enamel hypoplasia and periostitis in an 18th and 19th century English skeletal collection. The findings showed that individuals from high SES were less likely to exhibit lesions of cribra orbitalia than those from low SES (Yaussy, 2019). Similarly, Casna and Schrader (2022) assessed the prevalence of non-specific markers of physiological stress between low, middle-low, middle-high, and high SES groups of three different Dutch skeletal collections (1626 – 1850 CE). The results indicated statistically significant differences between low and high SES groups for the prevalence of cribra orbitalia (Casna and Schrader, 2022). However, for porotic hyperostosis and linear enamel hypoplasia, differences in prevalence were observed particularly between the middle-class and low SES groups (Casna and Schrader, 2022). A convoluted relationship exists between health, SES, and sociocultural conditions, highlighting the need for research to better understand this relationship, particularly in contemporary populations (Casna and Schrader, 2022).

1.3. The South African context

South Africans represent a highly heterogenous population, shaped by a complex history of colonialism, migration, and institutionalised racism. The South African population consists of four main groups, namely black, white, indian, and coloured (a distinct self-identified racial group) (Statistics South Africa, 2022). Krüger et al. (2018) provides information on the South African population and histories of the different population groups. The Apartheid regime (1948 – 1994) in South Africa entrenched socio-economic disparities, exacerbating inequalities and fostering division among communities based on race. Apartheid was a system of institutionalised racial segregation that disproportionately allocated government resources in favour of white South Africans to the detriment of black, indian, and coloured South Africans (Linford, 2011). Apartheid has contributed largely to disparities in SES among individuals within the country; as a result, access to high-level jobs, education, and residence in urban areas was restricted, leaving many individuals of colour without the resources required to improve their living conditions (Linford, 2017; Fogel, 2019). Presently, the economic effects of Apartheid are still prevalent in South Africa, even after its abolishment in the early 1990s (Mariotti and Fourie, 2014). As of 2022, 66,6 % of individuals in the population are living in poverty (World bank, 2023). As a result of multidimensional, generational poverty, many black and coloured South Africans continuously have inadequate access to health care and are highly susceptible to disease and the potential development of skeletal pathological lesions (Daniel, 2020; Stoddard, 2022).

Given the socio-political history in South Africa, the relationship between race and health outcomes has been a topic of significant discussion. In many societies, including South Africa, historical and systemic factors have led to disparities in health outcomes across racial groups. Race exists as a lived experience and an individual's racial designation (due to social constructs) can have enormous biological consequences pertaining to health and development (Mukhopadhyay, 2014; Ifekwunigwe et al., 2017; Wagner et al., 2017). Many researchers caution against the use of race as a variable in studies. In particular, research based on skeletal material has received criticism because of the sordid history of many bone collections and the acquisition of the remains in these collections (Morris, 1987; Morris, 2000; Legassick and Rassool, 2000; Rassool and Hayes, 2002; Steyn et al., 2013; Morris, 2014; Smith, 2015; Jansen and Walters, 2020; Ntatamala et al., 2023). This study aims to assess if stress, poverty, and poor health linked to historic racism and Apartheid have an impact on skeletal growth and formation among South Africans. Ultimately, this study cannot discuss SES and poverty without taking into consideration colonialism, Apartheid, and race due to its deeply entrenched history in the SES of the South African population, both historic and contemporary. Understanding these disparities and their underlying causes is crucial for addressing health inequities and promoting health equity for the purpose of redress.

1.4. Research aims and hypotheses

This study aimed to assess the frequency of four non-specific signs of physiological stress (i.e., cribra orbitalia (CO), porotic hyperostosis (PH), enamel hypoplasia (EH), periostitis (PR)) and its correlation to cranial FA in a South African sample. The cranium was selected for the assessment of FA as it is less affected by confounding factors such as side-bias (directional asymmetry) and muscular development. Since the pathogenesis of CO, PH, EH, and PR may be linked to developmental stress, these lesions are ideal to assess disease and FA in a South African sample. Based on findings in the literature, the following hypotheses were tested:

1. Black South Africans are hypothesised to demonstrate higher levels of FA compared to white South Africans, due to the difference in SES and developmental stressors;
2. Black South Africans are hypothesised to demonstrate higher prevalence of non-specific signs of physiological stress than white South Africans, due to the differences in SES and developmental stressors;
3. A correlation exists between non-specific signs of physiological stress and levels of FA in the cranium.

2. Materials and methods

2.1. Sample

The sample consisted of 115 crania (dry bones and micro-XCT scans) and their associated post-crania (dry bones) (Table 1), sourced from the Pretoria Bone Collection (PBC), housed at the University of Pretoria, South Africa. Only well-preserved crania (without metal restorations) and long bones were selected. The sample comprises South Africans born between 1863 and 1996 and thus includes individuals that grew up and were alive during the Apartheid regime (L'Abbé et al., 2005; L'Abbé et al., 2021). Although no specific information regarding individual living circumstances, lifestyle or overall health is known, the individuals housed in the PBC are associated with the current socio-political and socio-cultural landscape of South Africa (L'Abbé et al., 2021). The skeletal remains were obtained from cadavers of identified (but unclaimed by family members and not wilfully donated) and donated bodies that were acquired by the University of Pretoria medical school through informed consent by hospitals and the Director General (L'Abbé et al., 2005; Morris, 2007; L'Abbé et al., 2021). The unclaimed individuals typically represent individuals from lower SES as they are impoverished and are unable to afford burials. Thus, they are likely to have experienced physiological stress due to poverty (Tal and Tau, 1983; Ntatamala et al., 2023).

To explore differences in the prevalence of the conditions, the sample included male and female black and white South Africans, with all individuals older than 18 years of age (mean age-at-death: 53 ± 16 years). The median birth and death years of this sample range from 1943 to 1996 (min-max birth years: 1882–1980, min-max death years: 1964–2012). For black South African individuals, the median birth and death years are 1950–1996 (min-max birth years: 1882–1977, min-max death years: 1964–2011), while for white South African individuals, the median birth and death years are 1934–1996 (min-max birth years: 1896–1980, min-max death years: 1973–2012). Thus, indicating that the sample analysed in this study grew up and lived during the Apartheid period. Given the population history and individual birth/death dates, the black South Africans in the sample are presumed to represent individuals of lower SES, while the white South Africans are presumed to represent individuals of middle to higher SES.

Table 1
Study sample distribution: number of individuals (n) per sex and population groups, and median age (in years) of the individuals.

	Black South Africans	White South Africans	Total
Females *(n)*	30	27	57
Males *(n)*	29	29	58
Total *(n)*	59	56	115
Median age (minimum-maximum) (years)	41 (22–87)	62 (21–89)	51 (21–89)

While the assessment of pathological lesions was performed on dry bones (crania and post-crania), FA was evaluated on micro-XCT scans of the crania. The individuals were randomly selected from the available scanned crania in the *Bakeng se Afrika* virtual repository (L'Abbé et al., 2021), and thus may not necessarily be representative of the entire South African population. Micro-focus X-ray Computed Tomography (micro-XCT) imaging has shown to be extremely effective in providing detailed visualisation of facial structures that assists in identifying facial asymmetry without causing damage or destruction to skeletal material (Akhil et al., 2015). However, the examination of pathological conditions could not be done on micro-XCT scans because full-body micro-XCT scans were not available in the repository and pathological lesions were not easily visible on scans.

Ethical approval for this study was obtained by the Faculty of Health Sciences Research Ethics committee at the University of Pretoria (386/2021).

2.2. Methods

2.2.1. Fluctuating asymmetry (FA)

All cranial micro-XCT scans were first segmented using the semi-automatic watershed method in the Avizo 2019 software (ThermoFisher Scientific Inc.), followed by the re-orientation and standard alignment according to the Frankfort plane of the 3D models generated. A total of 15 interlandmark distances (ILD) were obtained from 34 landmarks placed on these cranial micro-XCT scans using Avizo 2019 (Fig. 1). Table 2 details the definitions of the landmarks and distances included in the study.

Several calculations were complete to quantify FA. First, FA values were calculated for each individual by subtracting the right-side measurements from the left-side measurements. Standard FA formulae (FA8 and FA17 indices) developed by Palmer and Strobeck (2003) were applied to take size into consideration (Formula 1 and 2). FA8 indices (the difference between the natural logs of the left and right measurements of a trait) were first calculated for each ILD, followed by FA17 indices, which are combinations of different FA8 indices for each specimen (Palmer and Strobeck, 2003, Hagg et al., 2017) (Formula 1 and 2). Five FA17 combinations were calculated (see Table 3).

Formula 1

$$FA8 = |\ln(R_d/L_d)|$$

FA8: the difference between the natural logs of the left (L_d) and right(R_d) distance (d) of a trait, in absolute values.

Formula 2

$$FA17 = \sum|\ln(R_d/L_d)|/\ T$$

FA17: the average FA values computed from the FA8 values for multiple traits and combinations. T is the total number of traits per index.

2.2.2. Pathological lesions

The crania and long bones (humeri, ulnae, radii, femora, tibiae, and fibulae) of the same individuals were all examined for pathological lesions typically linked to non-specific signs of physiological stress, anaemia, and/or nutritional diseases. Diagnostic aids, such as palaeopathological textbooks were used to effectively analyse the pathological lesions (Waldron, 2009; Ortner, 2012). Specifically, the presence of cribra orbitalia (CO) (Fig. 2), porotic hyperostosis (PH) (Fig. 3), enamel hypoplasia (EH) (Fig. 4) and subperiosteal bone reaction (PR) (Fig. 5) were recorded. CO was recorded as present if there were any porous

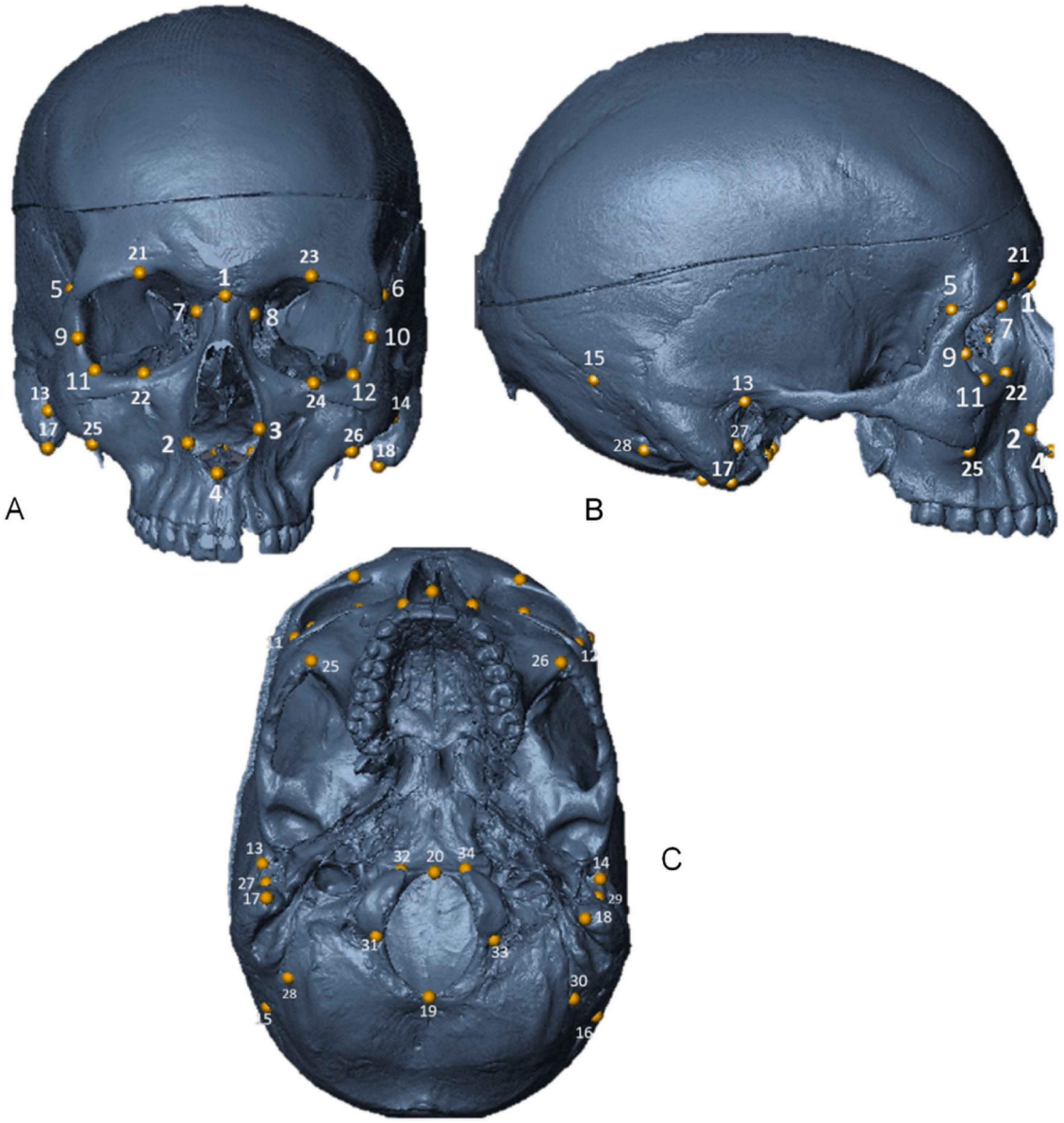

Fig. 1. Landmarks (1 – 34) placed on the cranium (cf. Table 2 for the names and abbreviations). A: Frontal view; B: Lateral view; C: Inferior view.

lesions on the roof of the orbits. Similarly, PH was considered as present if there were any porous lesions observed on the cranial vault. EH was recorded as present if at least one hypoplastic defect was observed on at least one tooth. PR was considered present if they were observed on at least one long bone. The pathological lesions were recorded as present regardless of whether the lesion appeared active or healed/in the process of healing. The lesion location and the number of lesions observed on each individual were also noted.

2.3. Statistical analysis

All statistical analyses were conducted in the R software and RStudio environment (R Core Team, 2016.

2.3.1. Repeatability testing

Repeatability testing was performed in several ways by assessing: (1) reproducibility of the landmark positioning; (2) technical error of measurement; (3) repeatability of the between-sides variation; and (4) observer agreement in recording pathological lesions.

First, the reproducibility of the landmark positioning (intra- and inter-observer agreement) was completed by placing the landmarks on 15 randomly selected scans. For the intra-observer agreement, landmark placement was performed twice by the same observer with an interval of two weeks. For the inter-observer agreement, an additional observer performed the landmark placement. The reproducibility of the landmark positioning was calculated using the dispersion (in mm) for each landmark and individual (Formula 3). Plots of dispersion were generated to show the variation of dispersion over different subjects.

Formula 3

$$\Delta_{ij} = \sum_{k=1}^{K} \left\| \boldsymbol{p}_{ijk} - \overline{\boldsymbol{p}}_{ij} \right\| \Big/ K, \text{ with } \overline{\boldsymbol{p}}_{ij} = \sum_{k=1}^{K} \boldsymbol{p}_{ijk} \Big/ K$$

Dispersion Δij*,defined as the Mean Euclidean Distance (MED) of the sample landmark* $\boldsymbol{p}ijk$ *to the mean* $\boldsymbol{p}$ *ij of the (x, y, z)-coordinates of landmark i over all observation's K (inter, intra, respectively) for subject j.*

Technical error of measurement (TEM) and relative technical error of measurement (%TEM) were calculated to evaluate the intra-observer error in left and right traits (i.e., ILDs) and for FA17. Additionally, two-way analysis of variance (*side* x *individuals* ANOVAs) were conducted with a Holm's adjustment to assess the between-sides variation

Table 2
Interlandmark distances calculated from the craniometric landmarks (adapted from Hagg et al., 2017). All interlandmark distances were calculated for left and right sides. Refer to Fig. 1 for the placement and numbering of landmarks. (Abr.: Abbreviation).

Name	Abr.	Definition
Orbital breadth	OBB	Distance from the dacryon (D, 7/8) to ectoconchion (Ec, 9/10).
Orbital height	OBH	Distance between the superior (So, 21/23) and inferior (Io, 22/24) orbital margins.
Diagonal orbital breadth	NOR	Distance from nasion (N, 1) to orbitale (Or, 11/12).
Frontomalare-nasion length	FMTN	Distance from frontomalare (Fmt, 5/6) to nasion (N, 1).
Frontomalare-nasospinale	FMTNS	Distance from frontomalaretemporale (Fmt, 5/6) to nasospinale (Ns, 4).
Malar height	MAH	Distance from the most inferior point on the lower border of the orbit (Or, 11/12) to the most superior point on the inferior border of the zygomatic (Z, 25/26).
Mastoid length	MPL	Distance from porion (Po, 13/14) to mastoidale (Ms, 17/18).
Mastoid breadth	MPB	Distance from the posterior border of the external auditory meatus (Pem, 27/29) to the most anterior point along the posterior margin of the mastoid process (Pm, 28/30).
Mastoidale-asterion	MSAST	Distance from mastoidale (Ms, 17/18) to asterion (Ast, 15/16).
Occipital condyle length	OCL	Distance from the most anterior (Aoc, 32/34) to the most posterior (Poc, 31/33) point on the occipital condyles.
Opisthion-porion length	OPO	Distance from the opisthion (O, 19) to porion (Po, 13/14).
Basion-porion length	BAPO	Distance from basion (Ba, 20) to porion (Po, 13/14).
Nasion-mastoidale	NMS	Distance from nasion (N, 1) to mastoidale (Ms, 17/18).
Nasion-alare	NAL	Distance from nasion (N, 1) to alare (Al, 2/3).
Nasospinale-alare	NAAL	Distance from nasospinale (Ns, 4) to alare (Al, 2/3).

Table 3
Definitions of the FA17 (the average FA values computed from the FA8 values for multiple traits and combinations) indices.

Indices	Definition
FA17	Average of all FA8 values.
FA17_Orbital	Average of FA8_OBH and FA8_NOR.
FA17_Face	Average of FA8_FMTN, FA8_FMTNS, FA8_MAH, FA8_NAL, and FA8_NAAL.
FA17_Temporal	Average of FA8_MPL, FA8_MPB, FA8_MSAST and FA8_NMS.
FA17_Base	Average of FA8_OCL, FA8_OPO and FA8_BAPO.

compared to measurement error, which confirms if FA is inflated by measurement error or not. The reliability of FA is influenced by the association between measurement error and the size difference between left and right sides (Fields et al., 1995). Therefore, to ensure the observed asymmetry was the result of more than just measurement error, the between-side variation needed to be significantly greater than measurement error (Palmer, 1994; Møller and Swaddle, 1997; Palmer and Strobeck, 2003). If the *side* x *individuals* interaction variance for each raw asymmetry score (MS_{int}) is not significant ($p<0.05$) relative to measurement error, then the measurement may be removed to yield more accurate asymmetry estimates (Palmer, 1994).

Finally, Cohen's Kappa tests were performed to assess observer agreement for the recording of pathological lesions. Fifteen randomly selected specimens were analysed for the absence or presence of CO, PH, EH and PR.

2.3.2. Analysis of FA indices

Fluctuating asymmetry values were calculated for each trait and each individual to assess the presence of cranial FA (FA8 and FA17 indices, refer to Formula 1 and 2).

Shapiro-Wilk normality testing was conducted on the FA indices, leading to the selection of non-parametric tests. Mann-Whitney-Wilcoxon rank sum tests were conducted to analyse the level of asymmetry based on FA indices between populations, while Kruskal-Wallis rank sum tests were performed with Bonferroni corrections (to avoid familywise error) to further investigate if both sex and population groups have a greater effect on the expression of FA. Kruskal-Wallis rank sum tests with Bonferroni corrections were also used to investigate the affect of FA17 on age.

2.3.3. Analysis of pathological lesions

Analyses were conducted to evaluate the frequency and presence of pathological lesions in the South African sample. Frequency distributions were calculated for the presence of the pathological lesions (for each disease) in population groups, and for population and sex, simultaneously. Pearson's chi-squared tests were conducted in conjunction with the frequency distributions to test for significant differences between the groups. Frequency distributions were also calculated to examine the number of pathological lesions an individual presented with, over the entire sample for the populations, and population and sex, simultaneously. Kruskal-Wallis testing was then conducted to identify significant differences between the groups per the number of pathological lesions. Chi-squared *post-hoc* tests and *post-hoc* Dunn's tests were conducted with a Bonferroni correction in conjunction with the Pearson's Chi-square and Kruskal-Wallis tests, respectively.

2.3.4. FA indices and pathological lesions

Point-biserial correlation tests were performed to assess the association between the presence of pathological lesions and levels of asymmetry (FA) in a pooled sample. Kendall's Tau Rank correlations were also calculated to examine the relationship between levels of FA and the number of pathological conditions an individual had. Confidence intervals were also included to further explain the results; all interval values that included 0 supported the null hypothesis while those that did not include 0 rejected the null hypothesis and had a significant *p*-value.

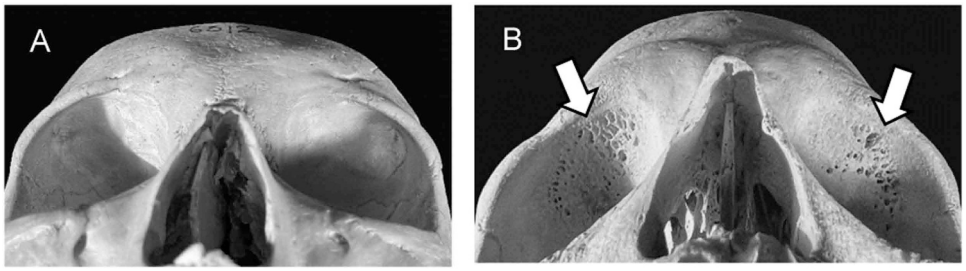

Fig. 2. Cribra orbitalia: A- unaffected individual (scored as absent) and B- Pitting present (white arrows).

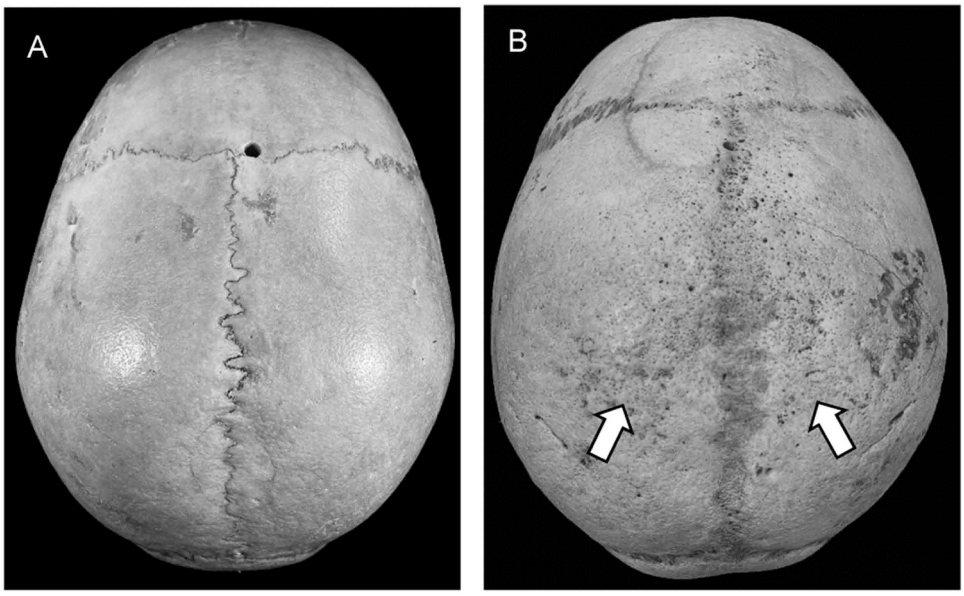

Fig. 3. Porotic hyperostosis: A- unaffected individual (scored as absent) and B- pitting present (white arrows).

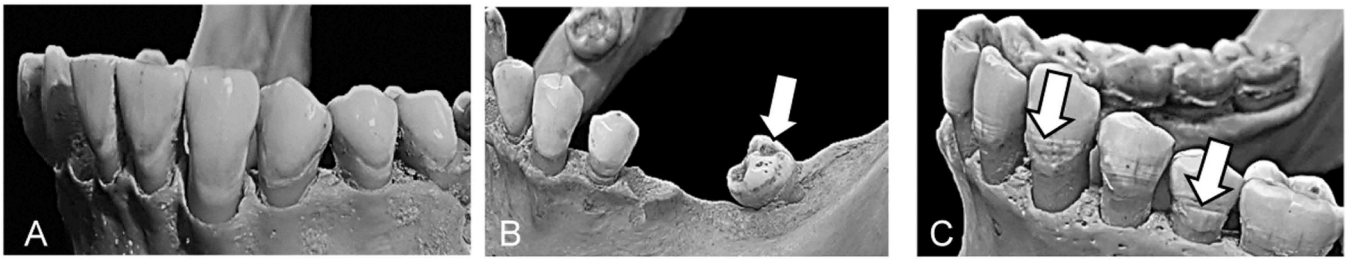

Fig. 4. Enamel hypoplasia: A- unaffected individual, B- spotted defects (white arrow) and C- linear defects associated with enamel hypoplasia (white arrows).

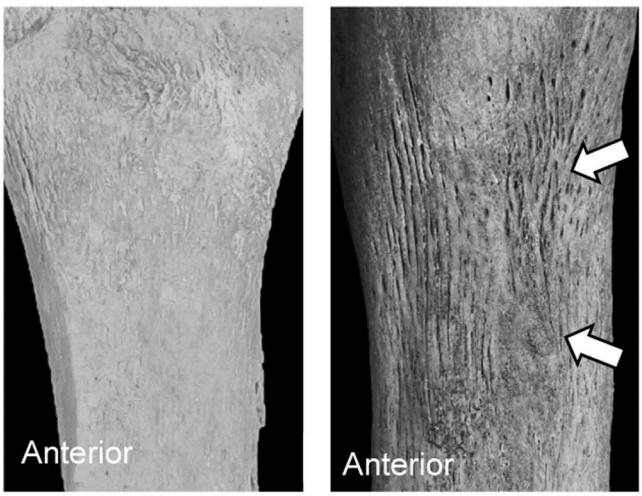

Fig. 5. Periostitis or subperiosteal bone reaction: A- unaffected individual, B- periostitis present (white arrows).

3. Results

3.1. Repeatability testing

Observer repeatability was tested for craniometric landmark placement to ensure accurate results, and that any differences in subsequent analyses were due to asymmetry rather than observer error. A threshold of 2 mm was chosen for measurement error as it is considered an acceptable amount of error in biological anthropology and accounts for normal asymmetric variation in crania (Stull et al., 2014; Choi, 2015).

For intra-observer error, the mean dispersion values ranged between 0.28 mm (SD ± 0.13) to 1.43 mm (SD ± 1.87), as illustrated in Fig. 6. All craniometric landmarks presented with a measurement error of less than 2 mm, showing high intra-observer reproducibility. For inter-observer error, the value ranged between 0.36 mm (SD ± 0.18) to 5.04 mm (SD ± 2.86). Majority of the craniometric landmarks presented with a dispersion error of less than 2 mm, while three landmarks, the right inferior orbital margin (Io, 22), and the right and left landmarks on the posterior margin of the mastoid (Pm, 28/30) fell above 2 mm (2.21 mm, 5.04 mm and 4.63 mm, respectively).

Intra-observer measurement error was assessed for individual traits (i.e., ILDs) using TEM and %TEM, which ranged between 0.45 and 1.48 mm, and 0.66–4.49 % respectively (Table 4). All traits fell within the acceptable range for a skilled observer assessing repeatability with themselves (1–5 %) (Perini et al., 2005). Given low dispersion error noted with the intra-observer analyses, and with all data being collected by the first author, the landmark placement and measurement error for individuals ILDs were considered acceptable.

Additionally, intra-observer TEM/%TEM for FA17, which represent a multivariate combination of ILDs, was calculated with low repeatability and high measurement error respectively (the larger the repeatability, the smaller the measurement error is relative to FA) (Table 4). Therefore, half the variation in FA17 is due to measurement error, which is usually the case with studies looking at cranial asymmetry. The level of measurement error noted could impact any patterns of statistically

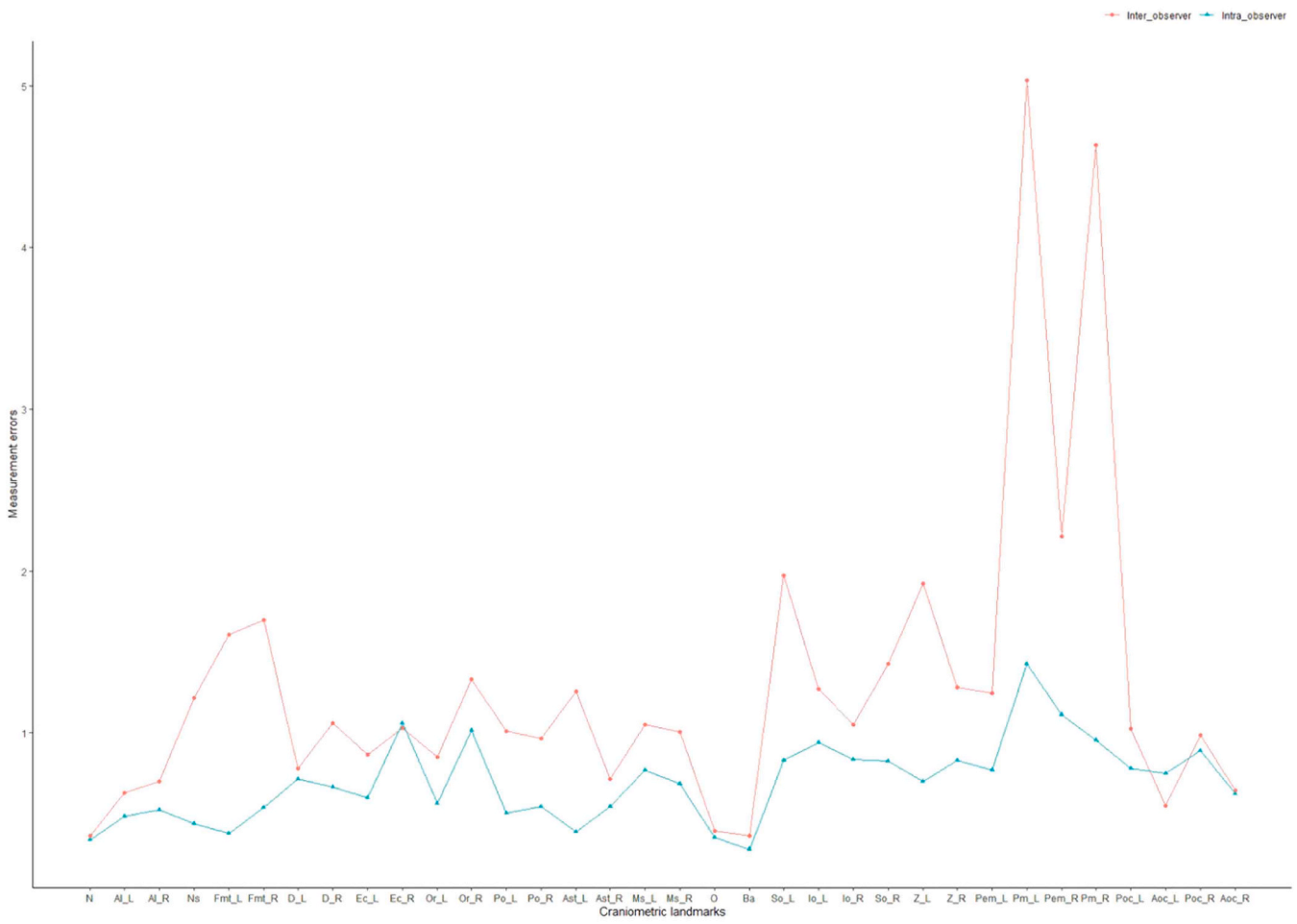

Fig. 6. Intra- (in blue) and inter-observer (in red) mean dispersion values (mm) for landmark positioning. Refer to Fig. 1 and Table 2 for the numbering and naming of the craniometric landmarks.

Table 4
Measurement error results from technical error of measurement (TEM, %TEM) values and two-way analysis of variance side x individuals (ANOVAs) for each trait (i.e., ILDs and FA17 indices). Bold indicates significance ($p < 0.05$).

Trait	N	TEM	Mean	%TEM	Side x Individuals ANOVA			
					df	MS_{int}	*F*	*p*-value
OBB	15	0.85	42.19	2.02	14	1.36	1.84	0.08
OBH	15	0.53	35.91	1.47	14	1.03	3.65	**0.00**
NOR	15	0.69	52.86	1.31	14	1.23	2.50	**0.03**
FMTN	15	0.47	56.00	0.85	14	1.21	5.33	**0.00**
FMTNS	15	0.49	75.01	0.66	14	1.25	5.07	**0.00**
MAH	15	0.63	20.81	3.04	14	2.33	5.84	**0.00**
MPL	15	0.92	29.26	3.16	14	1.89	2.18	**0.04**
MPB	15	1.48	32.97	4.49	14	10.37	4.70	**0.00**
MSAST	15	0.73	50.00	1.45	14	20.36	38.48	**0.00**
OCL	15	0.72	20.66	3.49	14	2.30	4.43	**0.00**
OPO	15	0.80	72.59	1.10	14	4.72	7.32	**0.00**
BAPO	15	0.45	60.07	0.75	14	7.77	35.22	**0.00**
NMS	15	1.11	120.87	0.92	14	10.57	8.41	**0.00**
NAL	15	0.81	41.92	1.94	14	2.68	4.03	**0.00**
NAAL	15	0.63	17.46	3.59	14	3.36	8.54	**0.00**
FA17	15	0.14	0.27	50.36				

N = number of traits, df = degrees of freedom, $MS_{int} = MS_{interaction}$ (side x individuals mean squares), F = test statistic of two-way ANOVA.
Refer to Table 2 for trait names.

significant levels of asymmetry found in the study, meaning any asymmetrical differences observed may not solely be due to FA.

To further assess the effect of measurement error and repeatability of the size difference between left and right sides, two-way ANOVAs were conducted. The ANOVAs indicated that only one cranial measurement (OBB) exhibited higher measurement error relative to the between-sides difference due to FA, as indicated by no statistical significance. Thus, OBB was removed from further analyses (Table 4). This follows the practices in similar studies (Storm, 2009; Hagg et al., 2017), as recommended by Palmer and Strobeck (2003).

The intra-observer agreement for the scoring of pathological lesions ranged from good to nearly perfect (k=0.73 to k=1.00). Both CO and PR achieved an almost perfect agreement (k=1.00), while EH achieved an excellent agreement (k=0.87) and PH achieved an overall good agreement (k=0.73). The inter-observer agreement was slightly lower than the intra-observer agreement, ranging between moderate to good (k=0.57 to k=0.76). Scores for both CO (k=0.72) and PR (k=0.74) had an overall good agreement between the two different observers, while PH (k=0.57) and EH (k=0.60) achieved moderate agreement between observers.

3.2. FA levels in a South African sample

Non-parametric Mann-Whitney-Wilcoxon rank sum tests were selected and conducted to analyse the asymmetry based on FA indices between the population groups. No significant asymmetrical differences were noted between the population groups. To further assess FA,

Kruskal-Wallis tests (with Bonferroni corrections) were conducted between sexes and population groups simultaneously to ensure that pooling does not mask any asymmetry (Table 5). No asymmetrical significant differences were noted. The age distribution is evenly distributed between the population groups, with white South Africans being older (median = 62). A Kruskal-Wallis rank sum test was then conducted to evaluate the impact of age on asymmetry between the FA17 indices and age cohorts (20–40 vs 41–60 vs 61–90). No statistically significant differences were noted for FA17 when comparing the three age cohorts (p=0.35; X^2= 2.13).

3.3. Prevalence of pathological lesions in a South African sample

The results for the whole sample were broken down to examine if significant differences were observed for the presence of pathological conditions between population groups, and population and sex groups, simultaneously. Pearson's chi-squared test (Table 6) showed a statistically significant relationship between the populations for the presence of PH, with a higher prevalence in black South Africans compared to white South Africans. A statistically significant relationship was also noted between black and white South Africans for the presence of EH, with a greater occurrence in black South Africans. Furthermore, Kruskal-Wallis tests showed significant differences for disease frequency between population groups ($p = 0.04$), demonstrating that black South Africans are more likely to exhibit multiple pathological lesions simultaneously. No other statistically significant differences were detected between populations for CO and PR.

Lastly, Pearson's chi-squared tests were performed between the frequency of pathological lesions and sex/population groups (Table 7). Black South African males showed the highest frequency for porotic hyperostosis, which was further confirmed by a chi-square *posthoc* test with Bonferroni correction. No other statistically significant relationship was observed between population and sex groups for any of the other conditions. Kruskal-Wallis tests showed significant differences between black South African males and females, as males are more likely to present with multiple lesion simultaneously ($p = 0.003$). It should also be highlighted that black South African males exhibited the most pathological lesions among all subgroups.

Table 5
. Kruskal-Wallis tests (with Bonferroni correction) on FA8 (the difference between the natural logs of the left and right measurements of a trait) and FA17 (the average FA values computed from the FA8 values for multiple traits and combinations) indices between sex/population subgroups. Bold indicates significance (p <0.05).

Index_IL distance	Chi-squared (X^2)	Df	*p*-value
FA8_OBH	1.10	3	1.00
FA8_NOR	2.65	3	1.00
FA8_FMTN	6.72	3	1.00
FA8_FMTNS	3.16	3	1.00
FA8_MAH	2.01	3	1.00
FA8_MPL	5.74	3	1.00
FA8_MPB	4.74	3	1.00
FA8_MSAST	1.30	3	1.00
FA8_OCL	7.69	3	0.74
FA8_OPO	5.40	3	1.00
FA8_BAPO	3.37	3	1.00
FA8_NMS	1.27	3	1.00
FA8_NAL	2.32	3	1.00
FA8_NAAL	2.13	3	1.00
FA17	6.22	3	1.00
FA17_Orbital	3.51	3	1.00
FA17_Face	3.99	3	1.00
FA17_Temporal	8.84	3	0.16
FA17_Base	1.25	3	1.00

Chi-squared = Test statistic of Kruskal-Wallis test, df = degrees of freedom

Table 6
. Frequency of pathological lesions observed (A = absence, P = presence) in black and white South Africans. Last line: Pearson's chi-squared (X^2) tests results for the presence of pathological lesions vs. population affinity. Bold indicates significant differences between groups, p<0.05.

	Cribra Orbitalia (n = 115)		Porotic Hyperostosis (n = 113)		Enamel Hypoplasia (n = 92)		Periostitis (n = 114)	
	A	**P**	**A**	**P**	**A**	**P**	**A**	**P**
Black	43	16	33	26	26	29	29	29
White	43	13	42	12	27	10	27	29
	X^2= 0.07; df = 1; p = 0.79		X^2= 5.09; df = 1; $\mathbf{p = 0.02}$		X^2= 4.98; df = 1; $\mathbf{p = 0.03}$		X^2= 0.00; df = 1; p = 1.00	

n: number of individuals, X^2: Pearson chi-squared statistic, df: degrees of freedom

Table 7
. Frequency of pathological lesions (A = absent, P = present) in black and white South African females (BF, WF) and males (BM, WM). Last line: Pearson's chi-squared tests results for the presence of pathological lesions between the sex/population groups. Bold indicates significant differences between sex/population groups, p <0.05.

	Cribra Orbitalia (n = 115)		Porotic Hyperostosis (n = 113)		Enamel Hypoplasia (n = 92)		Periostitis (n = 114)	
	A	**P**	**A**	**P**	**A**	**P**	**A**	**P**
BF	23	7	22	8	14	15	16	14
BM	20	9	11	18	12	14	13	15
WF	21	6	22	4	13	4	13	14
WM	22	7	20	8	14	6	14	15
	X^2= 0.72; df =3; p = 0.87		X^2= 15.36; df = 3; $\mathbf{p = 0.00}$		X^2= 6.17; df = 3; p = 0.10		X^2= 0.31; df = 3; p = 0.96	

n: number of individuals, X^2: Pearson chi-squared statistic, df: degrees of freedom

3.4. Correlations between pathological lesions and fluctuating asymmetry

Point-biserial correlation tests, were performed to examine the relationship between the presence of pathological lesions and the level of asymmetry (FA17) (Table 8). No statistically significant correlations were detected with the entire sample pooled. Further examination was done using Kendall's Tau Rank correlations to assess the correlation between the level of asymmetry (FA17) and the number of pathological conditions; no statistically significant correlations were noted ($\tau = 0.05$, p-value = 0.61). According to Fig. 7, illustrating the level of asymmetry (FA17) in function of the number of pathological lesions in a pooled sample, individuals with at least two pathological lesions express a high FA17 index. While similar results are observed in females and white South Africans, males with no pathological lesions have a higher FA index and black South Africans with only one pathological lesion have a higher FA index compared to the rest of the sample (Fig. 7).

Table 8
. Point-biserial correlation tests for the entire sample pooled between the presence of pathological lesions and asymmetry (FA17), with CI. Bold indicates significance (p <0.05).

	Cribra orbitalia	**Porotic hyperostosis**	**Enamel hypoplasia**	**Periostitis**
Asymmetry	p = 0.94 CI (−0.19; 0.18)	p = 0.47 CI (−0.12; 0.25)	p = 0.40 CI (−0.26; 0.11)	p = 0.64 CI (−0.14; 0.23)

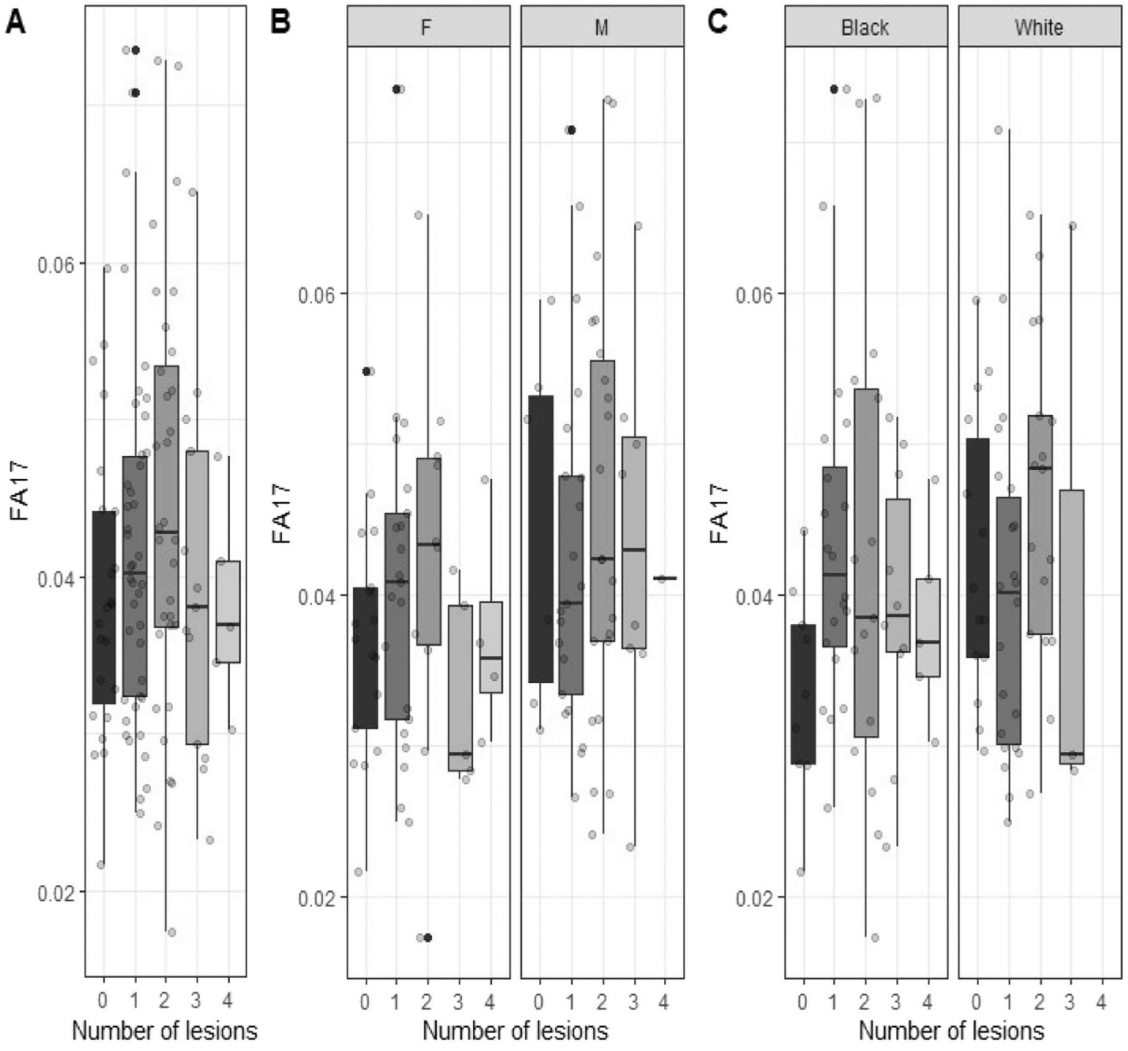

Fig. 7. FA17 (the average FA values computed from the FA8 values for multiple traits and combinations) in function of the number of pathological lesions in a pooled sample (A), in females (F) and males(M) (B), and between populations (C).

4. Discussion

4.1. Fluctuating asymmetry as an indicator of developmental stress

While it was hypothesised that black South Africans will show a higher level of cranial FA than white South Africans due to socio-economic differences, no significant differences were noted between the population groups. While this rejects our hypothesis, a possible explanation for this discordance may be due to measurement error, sampling and the osteological paradox. Given that half the variation in FA17 is due to measurement error, this could have masked or impacted the patterns of FA. In addition, the high %TEM could have inflated the likelihood of a type II error resulting in a false negative and failure to reject H_0 (No asymmetrical differences will be noted between population groups regardless of SES and developmental stressors). As discussed by Wood et al. (1992), sampling can influence the observations, particularly when assessing health from skeletal remains. The lack of asymmetrical differences between the population groups may be because the individuals in the sample did not live in abject poverty and thus did not experience disruptions in their growth and development. However, it could also be true that the disruptions (observed as asymmetry) may have remodelled throughout adulthood and were not quantifiable in the sample. The type of test chosen to evaluate significant levels of asymmetry may have also influenced our results. Kruskal-Wallis rank sum tests without Bonferroni corrections showed significant asymmetrical differences for FA17_Temp; conversely due to the sensitive nature of the Bonferroni corrections, our results were impacted. Furthermore, Bonferroni corrections are very conservative and can significantly reduce the strength of the Kruskal-Wallis tests (Kononenko and Kukar, 2007), resulting in our hypothesis being rejected unless the significant difference was relatively large. The findings suggest that population differences, particularly population and sex differences in terms of SES, does not have an association with the development of FA.

4.2. Prevalence of non-specific signs of physiological stress and social disparities

This study identified PR, PH, and EH, as prevalent skeletal indicators of physiological stress. In total, 51 % of individuals in the sample demonstrated PR, which is not particularly surprising, given the multitude of causes that may lead to the formation of this type of lesion. PR (also referred to as periostosis) often occurs due to inflammation of the periosteum which can develop due to stress and poor living conditions but may also be due to infectious and chronic disease or as a result of trauma or fractures (Waldron, 2009; Hagg et al., 2017). It is likely the case that many of the individuals that presented with periostosis suffered from other concurrent conditions. Additionally, PR may also occur at various stages of an individual's life and not just during childhood.

Ultimately, given that infectious disease is highly prevalent in the South African population (Boyles et al., 2019), regardless of SES, the presence of PR did provide some information of developmental stress affecting both populations.

Subsequently, a high prevalence of PH and EH was noted among black South Africans. PH, most commonly attributed to numerous factors like haemolytic or megaloblastic anaemia, vitamin C deficiency, parasitic infections, and genetic predisposition (Ortner, 2003; Fairgrieve and Molto, 2000; Brickley and Ives, 2008; Walker et al., 2009; Jatautis, Mitokaitė, and Jankauskas, 2011), was notably frequent in this population. High prevalence of nutritional deficiencies in black South African individuals is linked to multidimensional poverty, resulting in inadequate intake of essential vitamins like B9, B12, and C due to limited access to nutrient-rich foods and medical care (Gebremedhin, 2021; Makwela, 2017). The consumption of nutrient-poor, high-energy foods, such as carbohydrates and sugar, is reportedly common among black South Africans due to affordability and accessibility (Makwela, 2017). This dietary pattern, coupled with inadequate vitamin C and vitamin B12 absorption from carbohydrate-rich diets, increases the risk of both iron-deficient and megaloblastic anaemia and subsequently, PH (Ling and Chow, 1953). Alblas (2019) and Zakai et al. (2009) report similar findings as they found that black South Africans showed a high prevalence of anaemia owing to malnutrition, parasite pathogens and poor SES, particularly in rural areas of South Africa (Limpopo, Mpumalanga, and KwaZulu-Natal) that are endemic for malaria. The forced settlement of families in malaria-prone regions during Apartheid has heightened the vulnerability to the disease (Black Homeland Citizenship Act of 1970). Malaria is linked to dyserythropoiesis mediated by cytokines and growth factors, leading to a functional deficiency of vitamin B12 (Aggarwal et al., 2011; Pradhan, 2009). As such, parasite pathogens and infection may also influence the prevalence of porotic hyperostosis. Incidences of childhood respiratory infection are high in South Africa, thus also potentially contributing to the porotic lesions observed in the adults of this sample (O'Donnell et al., 2020). Nutritional deficiencies may also be due to the effects of migrant labour practice, specifically in relation to the notable prevalence of PH observed among black South African males in the sample. In 2011, it was estimated that 60 % of migrants in South Africa were male, of which 71,6 % were black Africans (Ferraro and Weideman, 2020). Migrant labourers often have poor living conditions in overcrowded areas far from their families, are highly exploited, and experience undernutrition and crime (Mazibuko, 2000). This has also been reported by Alblas (2019) and Hens et al. (2019), where males showed a significantly higher prevalence of PH due to the roles played by sex hormones. High levels of testosterone have shown to decrease immune function, thus increasing susceptibility to illness and infection, which may also play a role in the development of porotic lesions in the male crania (Graham and Özener, 2016). Thus, a combination of factors could be contributing to the prevalence of PH. However, more research is required to better explore the causes and comorbidities that may lead to the development of these porotic lesions in the South African population. Similar to PH, EH was frequently observed in the entire sample. EH is linked to nutritional stress, infection, disease, and endocrine dysfunction during ontogeny ((Waldron, 2009), and interrupt the normal formation of enamel resulting in defects, such as pits and lines on the enamel surface. Children raised in low socio-economic areas are likely to show signs of EH as a result of nutritional deficiencies and their living conditions. EH also has longevity into adulthood, as enamel cannot be replaced or remodelled once it has formed (Nikita, 2017). Consequently, given the population history and possible dire living conditions of individuals in this sample during Apartheid, black South Africans showed a high prevalence of enamel hypoplasia, as expected.

In conclusion, the following study found a greater prevalence of non-specific signs of physiological stress in individuals from lower socio-economic backgrounds (black South Africans) than those from higher socio-economic backgrounds (white South Africans), supporting our hypothesis and what has been observed in literature as discussed in the introduction.

4.3. Relationship between cranial FA and non-specific signs of physiological stress

Cranial FA was not significantly correlated to the presence or number of pathological lesions, rejecting our hypothesis. It should be acknowledged that lesions relating to CO, PH and PR can often heal due to the plasticity and remodelling of bone, and thus not being observable in adult individuals who have a high FA index. Cranial FA may have been more effective in capturing the severity of chronic stress as opposed to the presence of pathological lesions. Other explanations include the fact that nutritional deficits may not always produce skeletal lesions, or that individuals could have passed on before skeletal signs of physiological stress were able to develop. While we did not see a correlation between cranial FA and skeletal markers of stress, black South African males still exhibited a significant high level of cranial FA and high prevalence of non-specific signs of physiological stress.

This study provided more clarity on the variability of the expression of FA and skeletal indicators of stress in a contemporary South African sample. Moreover, the study noted the implications SES issues can have on health, and its overall reflection on skeletal development.

4.4. Osteological paradox

The osteological paradox is usually discussed in paleopathological studies focusing on archeological samples but should be acknowledged in any study addressing health from skeletal remains (Wood et al., 1992; DeWitte and Stojanowski, 2015; Hagg et al., 2017). With regard to the current study, the skeletal collection, from which the sample was selected, may have exhibited hidden heterogeneity, thus each individual likely has different levels of frailty within the population, which is often not known (DeWitte and Stojanowski, 2015). In turn, the FA levels in this study may have also been slightly more elevated compared to FA levels of the entire population due to the nature of the skeletal collections and the individuals that tend to form part of the collections. High levels of FA also decrease levels of fitness (Clark, 1995), thus increasing the risk of death leading to the elevated levels of FA in the sample. Subsequently, since this is an adult sample, the FA noted in the population may be due to more than just developmental instabilities and could be attributed to various other factors pertaining to lifestyle. In addition, for this sample specifically, hidden heterogeneity should be considered for the interpretation of pathological lesions given that few pathological lesions were observed in either population groups. Consequently, many individuals in this collection (and thus this sample), may have died due to illness or disease (related or unrelated to stress) before skeletal signs of physiological stress and pathological lesions could develop. Alternatively, pathological lesions will also not be present if individuals did not experience any stress or had a stronger immune system allowing them to buffer stressors (Hagg et al., 2017). Evidence of pathological lesions in this sample may be indicative of surviving early life stressors due to adaptive plasticity. A further consideration of age-at-death is needed in paleopathological and anthropological studies as older individuals or samples with pathological lesions may show low frailty and high resilience. However, those who survive to older ages without any lesions will result in a sample with mixed resilience and/or frailty. Therefore, the frequency of pathological lesions within this sample are not necessarily an accurate representation of physiological stress due to socio-economic circumstances within the population. This sample may be underrepresented and have mixed levels of frailty and resilience as lesions may not have been present on individuals who could have died from certain diseases and due to the variability of age-at-death of the adults in this sample.

4.5. Limitations

Possible limitations of this study include measurement error, and the lack of information on the life history and medical record of the individuals in this sample. With regard to measurement error, MPB showed the highest TEM and %TEM as well as variability in landmark placement. While the margin of error for MPB did fall within an acceptable range, caution should still be employed when using MPB definitions as landmarks are difficult to place. Moreover, multiple measurements or landmark placements can be done, and the overall average can be employed or MPB definitions can be modified. Due to half the variation in FA17 being due to measurement error as well, stricter protocols need to be employed in asymmetrical studies to ensure true patterns of FA are noted. Age was not analysed alongside pathological lesions, as the sample distribution did not have equal age ranges especially between sexes and populations groups. Thus, any analysis done on age would skew the data and would not provide accurate information on the overall impact of age on skeletal signs of physiological stress. The lack of information on life history and medical record of individuals does not give us the full view of the stressors experienced by the sample and therefore could have an impact on our interpretations.

5. Conclusion

The main aim of this study was to explore cranial fluctuating asymmetry and its relationship with non-specific signs of physiological stress in a South African skeletal sample, with individuals from different socio-economic backgrounds.

The main conclusions are as follows:

- Black South Africans did not show significant higher levels of FA compared to white South Africans.
- Non-specific signs of physiological stress for PH and EH were more prevalent in black South African individuals, with PH prevalent in black South African males as a result of nutritional deficiencies linked to socio-economic conditions. However, CO and PR had similar prevalence in both black and white South African populations.
- Levels of cranial FA were neither correlated to the presence or to the number of pathological lesions an individual had.
- Both population history, SES, and sex have no influence on cranial FA but may have a link to the expression of diseases.

CRediT authorship contribution statement

M. Harripershad: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **C.E.G. Theye:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **A.F. Ridel:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **L. Liebenberg:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

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