A comparative study of craniofacial measurements between Ryukyuan and mainland Japanese females using lateral cephalometric images

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Abstract Using lateral cephalometric images, we compared the skeletal and soft tissue configurations of Ryukyuan and mainland Japanese females. We collected lateral cephalometric images of 30 females each from Okinawa Island and mainland Japan. Sixty landmarks were plotted on each image. Then, based on the coordinates of the landmarks, 68 distances and 34 angles were calculated according to orthodontic and anthropometric methods. We confirmed that the Ryukyuans have a smaller height in the upper and midfacial region than the mainland Japanese. Moreover, our findings indicate that, compared with the mainland Japanese females, the Ryukyuan females clearly have the following features: (1) a shallower mandibular notch, (2) an anterior-inclined symphysis of mandible, and (3) a smaller depth from upper lip to incisors. We also found that an anterior-inclined mandibular corpus and incisors are associated with a smaller distance between the surfaces of the upper lip and teeth and with a more protruded lip shape.

Key words: cephalometry, facial morphology, Ryukyuans

Introduction

Physical anthropologists have investigated variation and diversity in human craniofacial morphology (Martin, 1928; Howells, 1973; Farkas, 1994); in such studies, skulls or living bodies were directly measured based on anthropometry. The craniofacial morphology of ancient and modern people in the Japanese archipelago has been well studied (von Baelz, 1911; Ikeda, 1974; Brace and Nagai, 1982; Hanihara, 1991; Ishida, 1992; Pietrusewsky, 1999; Dodo et al., 2000; Higa et al., 2003; Fukumine et al., 2006; Haneji et al., 2007; Toma et al., 2007; Fukase et al., 2012; Miyazato et al., 2014). Such studies have revealed that craniofacial features, including dental characteristics, show regional differences even within Japan, especially between the mainland Japanese, Ryukyu Islanders, and Ainu. These features also vary with the time period; differences between two ancient periods, the Jomon and Yayoi eras, are particularly pronounced. Therefore, physical anthropologists have proposed that the origin of the modern Japanese people has a dual structure; specifically, they have proposed that Jomon hunter-gatherers were the native inhabitants of the Japanese archipelago, and migrations of Yayoi farmers resulted in admixture between the two populations with the proportions of admixture varying among local regions (Hanihara, 1991). This model has been supported by genetic studies (Omoto and Saitou, 1997; Yamaguchi-Kabata et al., 2008; HUGO Pan-Asian SNP Consortium, 2009; Jinam et al., 2012; Koganebuchi et al., 2012; Sato et al., 2014).

Recent intensive research on the facial morphology of ancient and contemporary Ryukyu Islanders using 3-D digitizers and scanners has revealed detailed facial features. Fukase et al. (2012) have pointed out that the crania of early-modern Ryukyu Islanders have, on average, a lower facial height, a broader interorbital space, and sagittally more curved nasal bones than those of the modern mainland Japanese. Based on 3-D facial surface images of living subjects, Miyazato et al. (2014) have found that the Ryukyuan people have more prominent glabella and nasal roots, as well as lower facial and nasal heights.

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For over 80 years, the cephalogram has been a standard tool for orthodontic research on facial growth patterns (Franchi et al., 2007; Buschang and Jacob, 2014) as well as for diagnosis of the degree of malocclusion, treatment planning, and assessment of treatment efficacy (Broadbent, 1931; Steiner, 1953; Ricketts, 1957; Xiong et al., 2013). Orthodontists have examined worldwide ethnic groups to establish the cephalometric norms for each group (Cotton et al., 1951; Nanda and Nanda, 1969; Richardson, 1980; Bacon et al., 1983; Bishara et al., 1990; Swlerenga et al., 1994; Hwang et al., 2001). The cephalometric features of Japanese have also been well studied and compared with those of other populations (Miura et al., 1965; Uesato et al., 1978; Miyajima et al., 1996; Alcalde et al., 1998, 2000; Hayashi et al., 2012; Ahsan et al., 2013). These studies indicate that some facial features are characteristics of Japanese; for example, Japanese have, on average, a larger facial height, more protruded labia, and a less prominent chin compared with European-Americans (Miyajima et al., 1996), and a smaller nasolabial angle even compared with Chinese (Gu et al., 2011). However, the cephalometric norms of the Ryukyu Islanders, which are fundamental information for local orthodontists, have not yet been reported even though their facial features are known to be different from those of the mainland Japanese as discussed above.

An advantage of using cephalometric images is that they enable researchers to observe both skeletal and soft tissues simultaneously in the same individual. However, the cephalometric landmarks and measurements used in orthodontics are not sufficient to analyze the entire craniofacial form, because they are mainly distributed around the maxillary and mandibular areas (Steiner, 1953; Ricketts, 1957). Here, we plotted not only cephalometric landmarks but also anthropometric landmarks on lateral cephalograms of Ryukyuan and mainland Japanese females. Then, we compared skeletal and soft tissue configurations between the two groups to identify craniofacial characteristics specific to the Ryukyu Islanders.

Materials and Methods

Subjects

According to the inclusion criteria described below, we selected lateral cephalometric images of 60 female subjects, which had been taken from 2001 to 2013. Of these participants, 30 individuals were from Okinawa Island, a main island of the Ryukyu Islands, who were patients at the Yamauchi Dental Clinic and Adventist Medical Center, Okinawa, Japan. The other 30 individuals were patients at the Showa University Dental Hospital, Tokyo, Japan, and lived in or near Tokyo. The ages of the subjects ranged from 18 to 45 years old (mean 26.1, SD 7.56) for the Ryukyu group and from 18 to 39 years (mean 24.2, SD 5.06) for the mainland Japan group; differences in age between the subsets were not significant ($P = 0.274$). The birth years of the subjects ranged from 1968 to 1994 for the Ryukyu group and from 1964 to 1988 for the mainland Japan group.

The inclusion criteria for each individual were as follows: (1) age 18 years or older; (2) all four grandparents born either in the Ryukyu Islands or on mainland Japan; 3) Angle class I; (4) overjet within 1–5 mm; (5) overbite within 1–5 mm; (6) no congenital anomaly, no significant facial imbalance, and no missing teeth except third molars; and (7) no previous prosthetic replacement and orthodontic treatment. This study was approved by the ethics committees of the University of the Ryukyus and Showa University. All subjects provided written informed consent to participate in this research project.

Cephalometric analysis

Lateral cephalometric radiographs were taken in a natural head position with teeth in maximal intercuspation. The radiographic magnification ratio was 1.1. Each radiograph was digitized and processed on a computer using image analysis software (ImageJ, http://imagej.nih.gov/ij/). We used the 60 landmarks shown in Appendix 1 and Figure 1; each landmark is defined by orthodontic fields (Downs, 1948; Steiner, 1953; Rickets, 1957; Miyashita, 1996) and/or by the Martin method used in physical anthropology (Bräuer, 1988; Knussmann, 1988). These landmarks were manually plotted on facial profiles by one researcher (the first author, T.Y.) in order to eliminate interobserver variation. Note that our measurements were different from the definitions of the Martin method because distances were enlarged 1.1 times and because some measurements were projected to the sagittal plane instead of direct. Based on the coordinates of the landmarks, 68 distances and 34 angles were calculated (Appendix 2).

Statistical analyses

For each of the 102 measurements, differences between populations were examined by Student's *t*-test with Welch's collection assuming unequal variances. Multiple comparisons were corrected using the Benjamini and Hochberg (B-H) method (Benjamini and Hochberg, 1995). Although this method was originally designed as a false discovery rate (FDR)-controlling procedure for independent test statistics, it has been shown to work well as a multiple comparisons correction even when test statistics have positive regression dependency (Benjamini and Yekutieli, 2001). To characterize the pattern of craniofacial profiles, principal component analysis (PCA) was carried out using the correlation coefficient matrix of the 102 measurements. The differences in PC scores between two groups were assessed via Student's *t*-test. In addition, we performed discriminant analysis based on the 102 variables with or without a stepwise procedure (P_{in}, 0.025; P_{out}, 0.05) to build models for distinguishing between the two populations. The cross-validated accuracy was evaluated with the leave-one-out method. These analyses were performed using IBM SPSS Statistics software.

Results

Univariate comparisons between populations

For all measurements, the means and standard deviations for the Ryukyuan group and the mainland Japanese group are listed in Appendix 3. Of the 102 measurements, 30 showed $P < 0.05$ in the statistical test of the difference between means of two populations. Measurement values were generally smaller for the Ryukyuan females than for the mainland Japanese females. Using the B-H method to cor-

Table 1. Craniofacial measurements that show a significant difference between populations after correction for multiple testing

#	Variables	Landmarks used*	Ryukyu		Mainland Japan		*t*-test *P* value
			Mean	SD	Mean	SD	
#3	Middle facial height	Dy[2–3]	54.12	3.83	58.34	2.55	6.8.E–06
#26	Incision superioris to upper lip	Dx[27–24]	11.62	1.78	13.11	2.07	0.0043
#40	Upper facial height	D[2–35]	72.96	4.63	76.23	2.85	0.0019
#47	Nasion–nasospinale height	D[2–39]	54.45	3.63	58.48	2.70	1.0.E–05
#48	Rhinion–nasospinale height	D[37–39]	33.92	2.76	35.63	1.83	0.0068
#49	Length of nasal bones	D[2–37]	24.72	3.41	29.09	2.87	1.6.E–06
#50	Orbitale–nasospinale height	Dy[6–39]	26.30	3.04	29.97	2.54	4.8.E–06
#60	Greatest depth of mandibular notch	D[L(42,46)–47]	9.51	2.84	12.11	2.89	8.9.E–04
#68	Inclination angle of nasal bridge	A[35–2–37]	19.18	5.51	23.51	5.23	0.0028
#76	Symphyseal angle	A[38–4–9]	91.14	6.21	86.47	5.34	0.0029
#78	Menton–infradentale–pogonion angle	A[4–38–10]	34.28	6.92	38.77	5.14	0.0061
#88	Physiognomic upper facial height	D[53–58]	84.25	5.06	88.31	3.71	8.3.E–04
#89	Nasal height	D[22–53]	59.59	4.26	63.60	3.03	1.0.E–04
#91	Nasal length	D[53–59]	48.49	3.53	53.47	3.74	1.8.E–06

* D[a–b], distance between a and b; Dx[a–b] and Dy[a–b], distance between a and b in the direction horizontal and vertical to the FH plane, respectively; L(a,b), line formed by a and b; A[a–b–c] and A[L(a,b)–L(c,d)], angle formed by a–b–c and two lines, respectively.

rect for multiple testing, 14 measurements cleared the FDR threshold of <0.05 ($P < 0.0069$) (Table 1). Notably, the between-group differences for length of nasal bones (#49) and nasal length (#91) showed the lowest *P* values. Together with a smaller inclination angle of nasal bridge (#68), it was suggested that nasal bones of Ryukyuan females are shorter in both vertical and anteroposterior directions compared with those of mainland Japanese females. Additionally, measurements in the upper facial and midfacial parts—including the middle facial height (#3), upper facial height (#40), nasion–nasospinale height (#47), rhinion–nasospinale height (#48), orbitale–nasospinale height (#50), physiognomic upper facial height (#88), and nasal height (#89)—were smaller for Ryukyuan females than for mainland Japanese females.

Based on measurement #26 (incision superioris to upper lip), the Ryukyuan females seemed to have a smaller depth from the upper lip to the incisors. As for mandibular form, the greatest depth of the mandibular notch (#60) was larger for mainland Japanese females than for Ryukyuan females. In contrast, the Ryukyuan females had a larger symphyseal angle (#76) and a larger menton–infradentale–pogonion angle (#78), suggesting they had a more anterior-inclined mandibular symphysis than the mainland Japanese females. To help visually understand the differences between the two groups, the average positions of some landmarks were plotted as shown in Figure 2.

PCA

Appendix 4 and Figure 3 show results of the PCA based on the 102 measurements. For each of the 19 top-ranking PCs, the eigenvalue exceeded 1.0, and these 19 PCs contributed approximately 90% of the variance. However, it is difficult to interpret all the 19 PCs since statistical artifacts can accumulate in lower-ranking PCs. Therefore, here we interpreted only the five top-ranking PCs. Each of them contributes more than 5% of the variance, and together they explained approximately 57% of all variance.

PC1 (eigenvalue, 19.3; contribution, 18.9%) could be interpreted as a size-related component, in which allometric shape changes could also be included in the PC. This PC was positively associated with most of the distance measurements, especially facial heights; therefore angles related to facial heights such as mandibular plane angle (#10), gonial angle (#12), and basion angle (#73) are also positively associated with PC1 (Appendix 4). In contrast, PC1 was negatively correlated with some angles such as the SNA angle (#13), SNB angle (#14), and nasion angle (#71), which indicates that an increased PC1 score is linked to a recession of both the maxilla and the mandible in relation to the cranial base. To examine the possibility that the negative covariation between the facial height and the bimaxillary protrusion is spurious due to a statistical artifact or a confounding effect of between-population variation, we observed the correlations within each group (Table 2). The Ryukyuan subjects exhibited significant negative correlations of total facial height with SNA and SNB angles, whereas the mainland Japanese subjects showed smaller and insignificant negative correlation coefficients. This indicated that the facial height and the bimaxillary protrusion covary, at least in the Ryukyu group.

PC2 (eigenvalue, 14.3; contribution, 14.0%) had highly positive associations with the facial angle (#7), Z angle (#30), menton' t-radius (#96), U1 to FH angle (#17), and profile angle of mandible (#79), but highly negative associations with the Y axis angle (#11), mandibular plane angle (#10), occlusal plane angle (#21), total profile angle (#63), and tooth angle (#66) (Figure 3A and Appendix 4). These findings indicated that a smaller PC2 score was associated with a more receded mandible and more inferiorly inclined occlusal and mandibular planes.

PC3 (eigenvalue, 10.4; contribution, 10.2%) was characterized by highly positive PC loadings in the mental angle (#77), lower lip protrusion (#23), angle of convexity (#8),

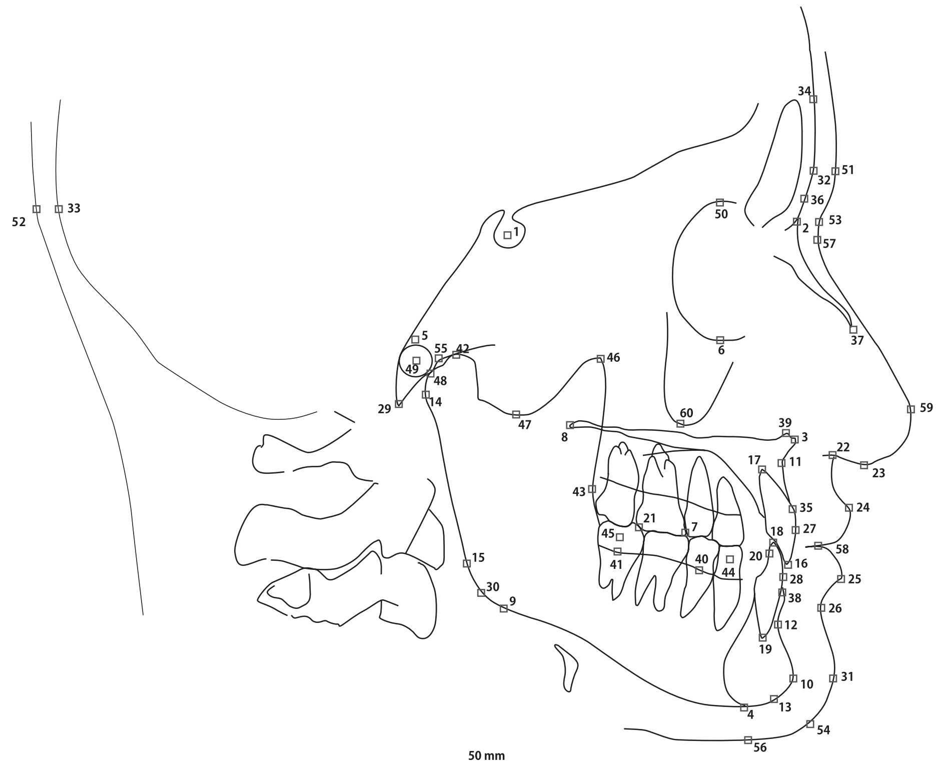

Figure 1. Landmarks plotted on each lateral cephalometric image.

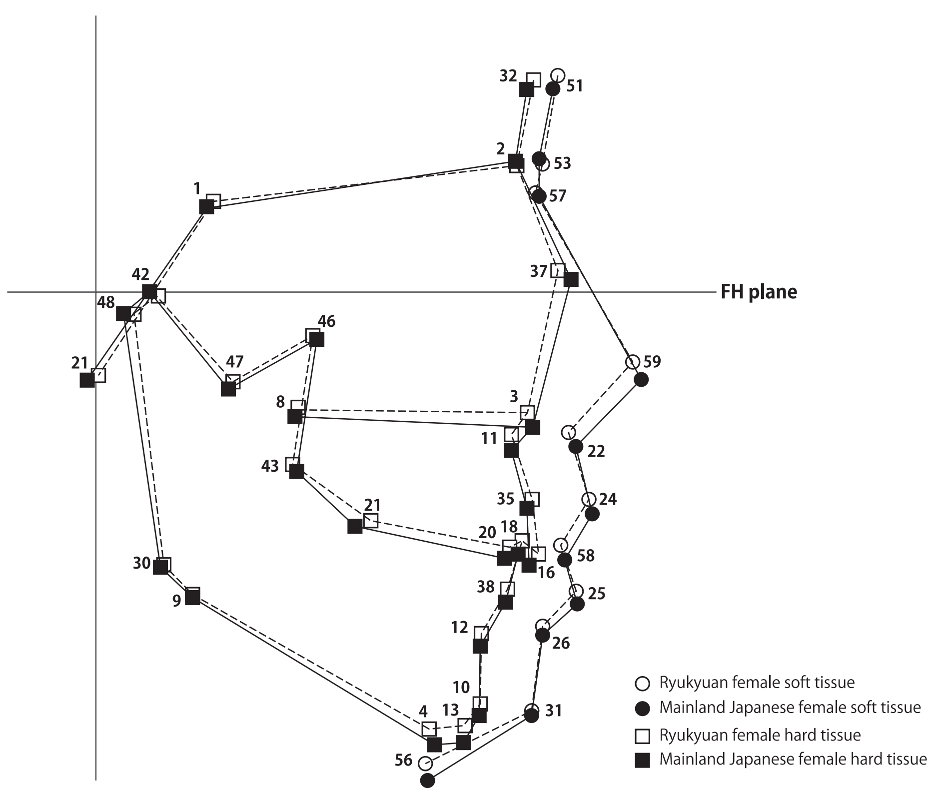

Figure 2. The average position of landmarks in each group

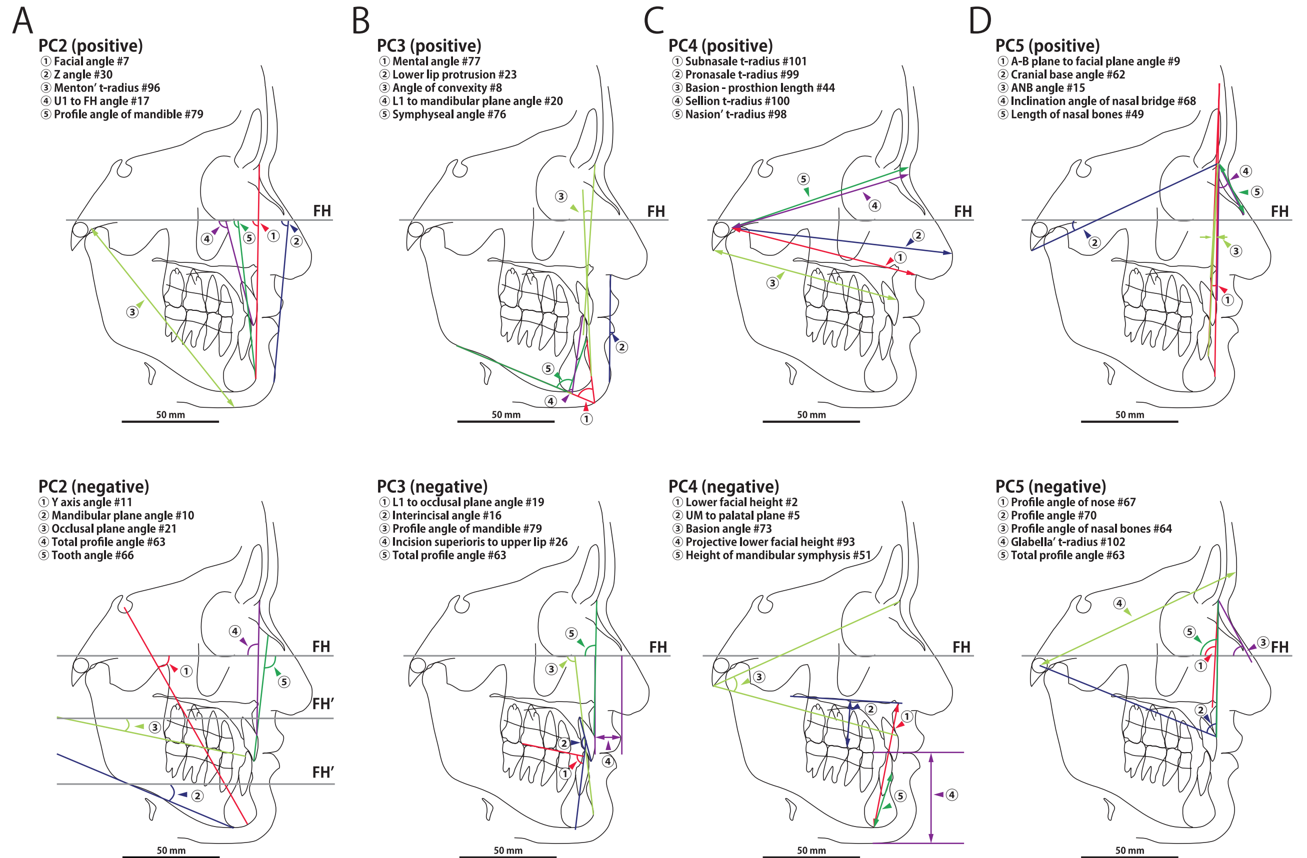

Figure 3. Measurements having highly positive or negative loadings to each PC. (A) PC2, (B) PC3, (C) PC4, and (D) PC5. PC1, which represents a size-related component, is not shown.

Table 2. Correlation between the facial height and the bimaxillary protrusion

		PC1	SNA angle #13	SNB angle #14
PC1	A	—	**–0.37**	**–0.39**
	R	—	**–0.43**	**–0.49**
	M	—	–0.24	–0.16
Total facial height #39	A	**0.94**	**–0.40**	**–0.40**
	R	**0.95**	**–0.48**	**–0.54**
	M	**0.90**	–0.22	–0.12

Correlation coefficients in all (A), Ryukyuan (R), and mainland Japanese (M) subjects are shown. Bold denotes a significant correlation.

Table 3. Correlation between the shapes of lips and mandibule

		PC3	Interincisal angle #16	Symphyseal angle #76	Mental angle #77
PC3	A	—	**–0.71**	**0.61**	**0.73**
	R	—	**–0.61**	**0.64**	**0.79**
	M	—	**–0.81**	**0.48**	**0.64**
Upper lip protrusion #22	A	**0.46**	**–0.44**	**0.28**	**0.54**
	R	**0.34**	–0.30	0.14	**0.50**
	M	**0.57**	**–0.55**	**0.39**	**0.60**
Lower lip protrusion #23	A	**0.69**	**–0.59**	**0.34**	**0.55**
	R	**0.64**	**–0.50**	0.32	**0.56**
	M	**0.76**	**–0.68**	**0.36**	**0.55**
Incision superioris to upper lip #26	A	**–0.57**	**0.36**	**–0.38**	**–0.30**
	R	**–0.52**	0.14	**–0.39**	–0.24
	M	**–0.54**	**0.50**	–0.18	–0.33

Correlation coefficients in all (A), Ryukyuan (R), and mainland Japan (M) subjects are shown. Bold denotes a significant correlation.

L1 to mandibular plane angle (#20), and symphyseal angle (#76) and by highly negative PC loadings in the L1 to occlusal plane angle (#19), interincisal angle (#16), profile angle of mandible (#79), incision superioris to upper lip (#26), and total profile angle (#63) (Figure 3B and Appendix 4). As a result, individuals with a smaller PC3 score bore an upright mandibular body and a sharp-angled chin. In addition, the shape of lips was associated with this PC. To consider the influence of between-population variation on this covariation pattern, we observed correlations between the shapes of mandible and lips (Table 3); significant correlations were detected on 'mental angle vs. upper/lower lip protrusion' and 'interincisal angle vs. lower lip protrusion' in both the Ryukyu and mainland Japan groups, on 'symphyseal angle vs. incision superioris to upper lip' in the Ryukyu group, and on 'interincisal angle vs. upper lip protrusion/incision superioris to upper lip' and 'symphyseal angle vs. upper/lower lip

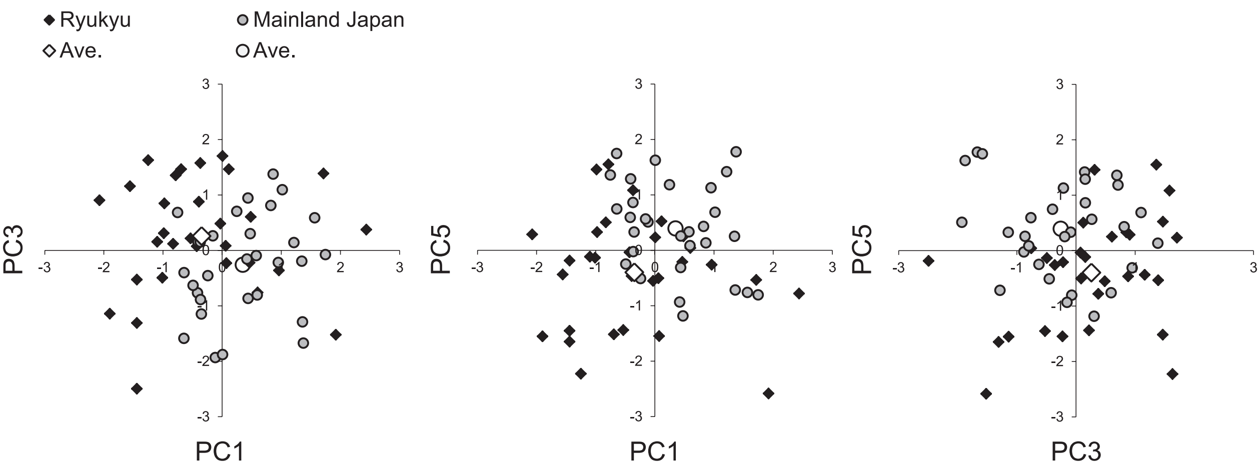

Figure 4. Two-dimensional scatter plots of PC scores.

protrusion' in the mainland Japan group. The results supported the presence of covariation between the shapes of mandible and lips within each group to some extent, although there may be an influence of population stratification in the analyses on all subjects.

PC4 (eigenvalue, 7.5; contribution, 7.4%) was positively associated with the subnasale t-radius (#101), pronasale t-radius (#99), basion–prosthion length (#44), sellion t-radius (#100), and nasion' t-radius (#98), but negatively with the lower facial height (#2), UM to palatal plane (#5), basion angle (#73), projective lower facial height (#93), and height of mandibular symphysis (#51) (Figure 3C and Appendix 4). Therefore, this PC was interpreted to reflect the relative depth of midface.

PC5 (eigenvalue, 7.0; contribution, 6.9%) could be considered a component related with the recession and protrusion of the upper face relative to the position of the lower part of the face. This component was positively associated with the A–B plane to facial plane angle (#9), cranial base angle (#62), ANB angle (#15), inclination angle of nasal bridge (#68), and length of nasal bones (#49), but negatively associated with the profile angle of nose (#67), profile angle (#70), profile angle of nasal bones (#64), glabella' t-radius (#102), and total profile angle (#63) (Figure 3D and Appendix 4).

The Ryukyuan females had significantly smaller PC1 (mean –0.35, $P = 0.0063$) and PC5 (mean –0.40, $P = 0.0016$) scores and a larger PC3 (mean 0.26, $P = 0.044$) score on average than the mainland Japanese females (Figure 4), while there were no significant differences in PC2 ($P = 0.35$) and PC4 ($P = 0.22$). In other words, a smaller midfacial height, an anteverted mandibular body, and a projected upper face were characteristic features specific to the Ryukyuan females, but these features are independent of one another. It is worth noting that data from the Ryukyuan females scarcely plotted to the fourth quadrant of the graph for PC3 vs. PC5 (Figure 4).

Discriminant analysis

Discriminant analyses were performed using all 102 measurements as explanatory variables. The cross-validated accuracies were 76.7–80.0% (Table 4). Discriminant analysis using the stepwise method identified only six variables, and the cross-validated accuracies using these six variables (90.0–96.7%) were higher than those using all the 102 variables (Table 4). The discriminant function was determined to be:

Table 4. Cross-validated discriminant accuracy

Region	102 measurements	Stepwise (6 measurements)*
Ryukyu	80.0%	90.0%
Mainland Japan	76.7%	96.7%

* Remaining independent variables: point A to subnasale (#25), length of nasal bones (#49), orbitale–nasospinale height (#50), condyle–coronoid length (#61), profile angle of nasal bones (#64), symphyseal angle (#76).

D = –2.542 + 0.188 [point A to subnasale] + 0.123 [length of nasal bones] + 0.235 [orbitale–nasospinale height] + 0.152 [condyle–coronoid length] – 0.085 [profile angle of nasal bones] – 0.113 [symphyseal angle],

where high (positive) and low (negative) D scores were associated with being mainland Japanese and Ryukyuans, respectively.

Discussion

Known and novel findings

In this study, it was confirmed that the Ryukyuan people have a smaller height in the upper and midfacial region compared with the mainland Japanese; it was also confirmed that the height of the lower face was almost identical between the two groups (Pietrusewsky, 1999; Dodo et al., 2000; Fukase et al., 2012; Miyazato et al., 2014). Less prominent nasal bones (rhinion) were observed in the Ryukyuan females; this finding corresponded to previous findings regarding male crania from the early-modern period where more detailed analyses had revealed that the Ryukyuans have nasal bones curved on the sagittal section (Fukase et al., 2012).

Although a study of 3-D facial surface images indicated that the Ryukyuans have a larger glabellar protrusion than the mainland Japanese (Miyazato et al., 2014), our findings did not detect this difference based on any single measurement, probably because we used different baselines and because females have less distinctive features than males. However, results from our multivariate analyses may have suggested such a characteristic in the Ryukyuans; specifically, the glabella' t-radius (#102) showed a relatively large negative correlation at a loading of –0.577 with PC5, and PC5 showed smaller scores in Ryukyuan females than in mainland females; notably, the loadings for nasion' t-radius (#98) and subnasale' t-radius (#101) were –0.385 and 0.057, respectively.

In this study, we elucidated several new findings using the metric data of both skeletal and soft tissues from the cephalograms based on both univariate and multivariate analyses. Compared with the mainland Japanese females, the Ryukyuan females clearly have the following features: (1) a shallower mandibular notch, (2) an anterior-inclined symphysis of the mandible, and (3) a smaller depth from the upper lip to the incisors.

Mandibular ramus

The Ryukyuan females had a significantly smaller greatest depth of mandibular notch (#60) and also a modestly smaller condyle–coronoid length (#61) ($P = 0.0086$) than the mainland Japanese, even though there was no difference in the projective length of ramus (#54) or coronoid height (#59) between the two groups. Reportedly, in the Japanese archipelago, the ramus breadth has decreased over time from the prehistoric period to the contemporary period, and geographically decreases from north to south (Kaifu, 1997; Fukumine et al., 2001; Maeda, 2002); the Hokkaido Jomon females had the widest ramus (37.5 mm), and the Ryukyuan females in the early-modern period had the narrowest (29.9 mm). Consistent with the previous findings, the minimum breadth of ramus tended to be smaller in the Ryukyuan females than in the mainland Japanese females in the present study ($P = 0.045$).

Mandibular corpus

Between-group differences in the anterior–posterior inclination of the symphysis could be assessed via two different methods: (1) measurements such as the symphyseal angle (#76) and the L1 to mandibular plane angle (#20); and (2) analysis of PC3. Based on the symphyseal angle (#76), Ryukyuans had a more anterior-inclined symphysis than the Japanese females. This conclusion was also supported by the marginal difference in the L1 to mandibular plane angle (#20) ($P = 0.024$). PC3 scores also showed that Ryukyuan females have such a characteristic. In contrast, the mainland Japanese females had a vertical symphysis and a sharp-angled mentum.

Lips

That the Ryukyuan females had a smaller depth from the upper lip to the incisors than the mainland females was suggested from a significantly smaller distance from the incision superioris to the upper lip (#26) and a modestly smaller distance from point A to subnasale (#25) in the Ryukyuan females ($P = 0.0077$). In addition, the distance from the incision superioris to the upper lip was strongly associated with PC3. This association indicated that the upper lip trait was not independent of the anterior inclination of mandibular symphysis and incisors. Moreover, the upper lip protrusion (#22) and lower lip protrusion (#23) were also strongly associated with PC3. All together, we found that an anterior-inclined mandibular corpus and inclined incisors were linked to a smaller distance between the surfaces of the upper lip and the teeth as well as to a more protruded lip shape. Miyajima et al. (1996) have reported that Japanese have more protruded labia and a less prominent chin than European-Americans. Similar differences were also observed between Mexican- and European-Americans (Vela et al., 2011). Our findings suggested that these labial and mandiblar characteristics are related to each other even within the Japanese population. It is likely that the shape of hard tissues changes the shape of external soft tissues. In contrast, lip thickness and strain reportedly have an influence on the relationship between dental and integumental tissue changes in orthodontically treated patients (Oliver, 1982). Therefore, it is possible that hard and soft tissue shapes affect each other.

Discrimination between the Ryukyuan and mainland Japanese females

We demonstrated that only six of the 102 measurements could efficiently discriminate between the Ryukyuan and mainland Japanese females. Each selected measurement represented population-specific features that could predict the membership of individuals. In the *t*-test comparing two groups, three of the six measurements showed a significant P value ($P < 0.0069$) (Table 1), another two showed $P < 0.05$, and the other one (profile angle of nasal bones #64) showed $P = 0.051$ (Appendix 3). The profile angle of nasal bones was highly related to PC5 (Figure 3), indicating that this measurement represented the facial futures expressed by this PC.

The discrimination accuracy using the one-leave-out cross-validation was surprisingly high (more than 90%). Miyazato et al. (2014) have reported that, as a result of a stepwise discriminant analysis based on 27 measurements of 3-D facial surface images, two variables that represent facial height and glabella protrusion can be used to assign an individual to the population of origin (Ryukyuan or mainland Japanese) with cross-validated accuracies of 80.0% (males) and 66.7% (females). Compared with the previous study, the present study resulted in more accurate prediction of population membership for each individual by means of comprehensive measurements that include both hard and soft tissues.

Genetic and environmental factors

In Japanese populations, the gonial angle increased and the height of the mandibular corpus decreased from the prehistoric times to the present (Kaifu, 1997). Since it has been generally hypothesized that the Ryukyuans received

Jomon-derived genes more than the mainland Japanese did, it would be interesting to see whether these mandibular traits were different between the two modern populations. In the present study, we observed a smaller average gonial angle (#12) in Ryukyuan females than in the mainland Japanese females, but the differences were not statistically significant. In the PCA, the variation in the gonial angle was associated especially with PC2, and the two groups did not differ significantly with regard to PC2. The height of the mandibular symphysis (#51) and the height of the mandibular body at 2nd premolar (#52) were also similar between the two groups, although the Ryukyuan females tended to have a larger height of the mandibular body at 2nd molar (#53) ($P = 0.016$).

These results may support the hypothesis that temporal changes in the mandibular morphology in Japan were due to environmental factors. Since all contemporary Japanese people, including Ryukyuans, consume a fairly similar variety of foods, based on an isotope analysis (Yoneda, personal communication); it is thus unlikely that the masticatory load on the mandible differs between local Japanese populations. In contrast, ancient people heavily depended on some specific foods (Naito et al., 2013), which would explain the temporal changes in the mandibular morphology. It has also been reported that the mandible, unlike the cranium, significantly reflects subsistence strategy rather than neutral genetic patterns; hunter-gatherers have generally longer and narrower mandibles than agriculturalists (von Cramon-Taubadel, 2011). On the other hand, morphological differences in Ryukyuan and mainland Japanese faces that we found in the present study would mainly be attributed to genetic factors.

Studies on animal models and human genetic disorders have led to increased knowledge about the processes of craniofacial morphogenesis and genes related to these processes (Wilkie and Morriss-Kay, 2001; Cohen, 2002; Kuratani, 2005; Radlanski and Renz, 2006). However, the common genetic factors that explain facial variation among human individuals or facial difference among populations remain unclear. Only a few genome-wide association studies (GWASs) have addressed human facial morphology; notably, single nucleotide polymorphisms (SNPs) in *paired box 3* (*PAX3*) are significantly associated with the shape of the nasal root in people of European descent (Paternoster et al., 2012; Liu et al., 2012). An association study focusing on 10 candidate SNPs shows that one SNP in *interferon regulatory factor 6* (*IRF6*), which is known as a risk factor for non-syndromic cleft lips/palates, affects normal variation in lip shape in Han Chinese females (Peng et al., 2013). Additionally, *growth hormone receptor* (*GHR*) polymorphisms are reportedly associated with mandibular ramus height (Yamaguchi et al., 2001; Tomoyasu et al., 2009). These polymorphisms may be involved in the variation in the shapes of lips and mandibles that we documented in this study. Dental morphology may affect facial morphology, and three polymorphisms in three genes—*ectodysplasin A receptor* (*EDAR*), *wingless-type MMTV integration site family, member 10A* (*WNT10A*), and *paired box 9* (*PAX9*)—can affect tooth size and non-metric dental traits (Kimura et al., 2009, 2015; Lee et al., 2012; Park et al., 2012). Recent population genomics studies have demonstrated a clear genetic differentiation between Ryukyuans and mainland Japanese (Yamaguchi-Kabata et al., 2008; HUGO Pan-Asian SNP Consortium, 2009; Sato et al., 2014), and a nonsynonymous SNP in *EDAR* is especially differentiated between the two populations. Therefore, this polymorphism may be a strong candidate for a causal gene responsible for morphological differences between Ryukyuan and mainland Japanese faces.

Currently, it is difficult to specify what factors generate the difference in congenital morphological features between populations. Previous studies on human crania have suggested that the morphological variation in the human skull has been largely shaped by neutral evolution, i.e. by genetic drift (Roseman and Weaver, 2007; Relethford, 2010). However, the variation in the nasal shape has been hypothesized to be a product of climatic adaptation, because nasal measurements are highly differentiated among populations and have a correlation with temperature and humidity (Thomson and Buxton, 1923; Roseman and Weaver, 2004). A recent study on the 3-D facial surface suggests that local adaptation and/or sexual selection have been important in shaping human soft-tissue facial morphology (Guo et al., 2014). The Ryukyuans and mainland Japanese are genetically similar. Nonetheless, we found clear morphological differences between the two populations. The population-specific facial features identified in the present study may become keys to understanding the processes of morphological differentiation among human populations.

Concluding remarks

Based on lateral cephalograms, we found several novel morphological differences in both hard and soft tissues between Ryukyuan and mainland Japanese females. Marked differences were found in the height of the upper face and the midface and the shape of the nose, mandibular ramus, symphysis of mandible, and lips. These findings will be foundational and helpful for orthodontic treatments of local people in Okinawa. However, we should recognize some limitations of this study. First, the subjects consisted of only females because we could not collect a sufficient number of male samples that met the inclusion criteria. Second, only lateral cephalograms could be analyzed in this study because the availability of frontal cephalograms was limited. To obtain robust results, further studies that use a larger sample size and include both males and females are required. Furthermore, a recent increase in availability of computed tomography and magnetic resonance imaging will elucidate morphological relationships between hard and soft tissues as well as craniofacial characteristics of the Ryukyuans in more detail. The present study provides the points to which we should pay attention in future research.

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Appendix 1. Sixty landmarks plotted on the lateral cephalogram in this study

No.	Landmarks	No.	Landmarks
1	Sella (s)	31	Pogonion' (pg')
2	Nasion (n)	32	Glabella (g)
3	Anterior nasal spine (ans)	33	Opisthokranion (op)
4	Menton (me)	34	Supraglabellare (sg)
5	Porion (po)	35	Prosthion (pr)
6	Orbitale (or)	36	Supraorbitale (so)
7	Mesial point of upper 1st molar	37	Rhinion (rhi)
8	Posterior nasal spine (pns)	38	Infradentale (id)
9	Posterior contact point for conprises a mandibular plane	39	Nasospinale (ns)
10	Pogonion (pg)	40	Alveolar process superior central border of lower premolar II
11	Point A (subspinale)	41	Alveolar process superior central border of lower molar II
12	Point B (supramentale)	42	Highest point of the condylar process
13	Gnathion (gn)	43	Mandibular ramus smallest width point
14	Mandibular ramus rear line (cd gathering)	44	Central point of lower premolar I
15	Mandibular ramus rear line (go gathering)	45	Central point of lower molar II
16	Upper incisor (the cut end)	46	Koronion (kr)
17	Upper incisor (apical)	47	Deepest point of mandibular notch
18	Lower incisor (the cut end)	48	Condylar process posterior extremity
19	Lower incisor (apical)	49	Earhole central point
20	Occlusal plane (icisor point)	50	Supraorbital margin middle point
21	Occlusal plane (molar point)	51	Glabella' (g')
22	Subnasale' (sn')	52	Opisthokranion' (op')
23	Septoculmion' (sec)	53	Nasion' (n')
24	Labrale superius' (ls')	54	Supragnathion' (sgn')
25	Labrale inferius' (li')	55	Tragion' (t')
26	Deepest point of mentale ditch	56	Menton' (me')
27	Surface of upper central incisor	57	Sellion' (se')
28	Surface of lower central incisor	58	Stomion' (sto')
29	Basion (ba)	59	Pronasale' (prn')
30	Gonion (go)	60	Zygomaxillare (zm)

Single quotation mark (') expresses a point on the soft tissue. For the detailed definition of the landmarks, see Miyashita (1996) and/or Knussmann (1988).

Appendix 2. One hundred and two measurement items in this study

#	Measurements	Landmarks used*	Martin's #**	Definitions
Orthodontics				
Hard tissue				
Distance				
#1	S–N plane	D[1–2]		Distance from sella to nasion
#2	Lower facial height	Dy[3–4]		Vertical distance from anterior nasal spine to menton
#3	Middle facial height	Dy[2–3]		Vertical distance from nasion to anterior nasal spine
#4	Full facial height	Dy[2–4]		Vertical distance from nasion to menton
#5	UM to palatal plane	D[7–L(3, 8)]		Distance from upper first molar mesial point to palatal plane
#6	LM to mandibular plane	D[41–L(4,9)]		Distance from lower first molar mesial point to mandibular plane
Angle				
#7	Facial angle	A[L(2,10)–L(5,6)]		Angle between facial plane and FH plane
#8	Angle of convexity	A[2–11–10]		Angle formed by nasion–point A–pogonion
#9	A–B plane to facial plane angle	A[L(2,10)–L(11,12)]		Angle between facial plane and point A–point B line
#10	Mandibular plane angle	A[L(4,9)–L(5,6)]		Angle between mandibular plane and FH plane
#11	Y axis angle	A[L(5,6)–L(1,13)]		Angle between FH plane and sella–gnathion line
#12	Gonial angle	A[L(4,9)–L(14,15)]		Angle between mandibular plane and mandibular ramus rear line
#13	SNA angle	A[1–2–11]		Angle formed by sella–nasion–ponit A
#14	SNB angle	A[1–2–12]		Angle formed by sella–nasion–ponit B
#15	ANB angle	A[11–2–12]		Angle formed by point A–nasion–ponit B
#16	Interincisal angle	A[L(16,17)–L(19,18)]		Angle between the line through the edge and root apex of upper central incisior and that of lower central incisor
#17	U1 to FH angle	A[L(16,17)–L(5,6)]		Angle between the line through the edge and root apex of upper central incisor and FH plane
#18	U1 to SN angle	A[L(1,2)–L(16,17)]		Angle between the line through the edge and root apex of upper central incisor and SN plane
#19	L1 to occlusal plane angle	A[L(20,21)–L(19,18)]		Angle between the line through the edge and root apex of lower central incisor and occlusal plane
#20	L1 to mandibular plane angle	A[L(4,9)–L(19,18)]		Angle between the line through the edge and root apex of lower central incisor and FH plane
#21	Occlusal plane angle	A[L(20,21)–L(5,6)]		Angle between occlusal plane and FH plane
Soft tissue				
Distance				
#22	Upper lip protrusion	D[L(22,31)–24]		Distance from pogonion'–subnasale' line to labrale superius'
#23	Lower lip protrusion	D[L(22,31)–25]		Distance from pogonion'–subnasale' line to labrale inferius'
#24	Labiomental sulcus	D[L(25,31)–26]		Maximum depth of labiomental sulcus from labrale inferius'–pogonion' line
#25	Point A to subnasale	D[11–22]		Distance from point A to subnasale
#26	Incision superioris to upper lip	Dx[24–27]		Projective length between labrale superius' to incision superioris on FH plane
#27	Incision inferioris to lower lip	Dx[25–28]		Projective length between labrale inferius' to incision inferioris on FH plane
#28	Pogonion to pogonion'	D[10–31]		Distance from pogonion to soft tissue pogonion'
Angle				
#29	Nasolabial angle	A[L(23–22–24)]		Angle between subnasale'– septoculmion' and subnasale'–labrale superius' lines
#30	Z angle	A[L(25,31)–L(5,6)]		Angle between labrale inferius'–pogonion' line and FH plane
Anthropometry				
Hard tissue				
Distance				
#31	Maximum cranial length	D[32–33]	[1]	Distance from glabella to opisthocranion
#32	Nasion–opisthocranion length	D[2–33]	[1d]	Distance from nasion to opisthocranion
#33	Basion–nasion length	D[2–29]	[5]	Distance from basion to nasion
#34	Basion–opisthocranion length	Dx[29–33]	[6(1)]	Projective distance from basion to opisthocranion on FH plane

Appendix 2. (continued)

#	Measurements	Landmarks used*	Martin's #**	Definitions
#35	Nasion–supraglabellare chord	D[2–34]	[29(1)]	Distance from nasion to supraglabellare
#36	Glabellar projection	D[L(2,34)–32]	[29h]	Maximum projective length from nasion–supraglabellare chord to median sagittal profile between the two points
#37	Supraorbitale–menton length	D[4–36]	[47b]	Distance from supraorbitale to menton
#38	Supraorbitale–prosthion length	D[35–36]	[48b]	Distance from supraorbitale toprosthion
#39	Total facial height	D[2–4]	[47]	Distance from nasion to menton
#40	Upper facial height	D[2–35]	[48]	Distance from nasion to prosthion
#41	Prosthion–menton height	D[4–35]	[48(2)]	Distance from prosthion to menton
#42	Alveolar field height	D[22–35]	[48(1)]	Distance from subnasale to prosthion
#43	Zygomatic field height	Dy[6–60]	[48(3a)]	Projective vertical length between orbitale and zygomaxillare
#44	Basion–prosthion length	D[29–35]	[40]	Distance from basion to prosthion
#45	Basion–menton length	D[4–29]	[42]	Distance from basion to menton
#46	Nasion–orbitale length	Dy[2–6]	[48(5)]	Projective vertical length between nasion and orbitale
#47	Nasion–nasospinale height	D[2–39]	[55]	Distance from nasion to nasospinale
#48	Rhinion–nasospinale height	D[37–39]	[55(1)]	Distance from rhinion to nasospinale
#49	Length of nasal bones	D[2–37]	[56]	Distance from nasion to rhinion
#50	Orbitale–nasospinale height	Dy[6–39]	[55(2)]	Projective vertical length between orbitale and nasospinale
#51	Height of mandibular symphysis	D[4–38]	[69]	Distance from infradentale to menton
#52	Height of mandibular body	D[L(4,9)–40]	[69(1)]	Distance from alveolar process to base of mandibular body at mental foramen
#53	Height of mandibular body (M2)	D[L(4,9)–41]	[69(2)]	Distance from alveolar process to base of mandibular body at 2nd molar
#54	Projective length of the ramus	D[L(4,9)–42]	[70a]	Projective vertical length from mandibular plane to highest point of the condylar process
#55	Minimum breadth of ramus	D[L(14,15)–43]	[71]	Minimum anteroposterior breadth of mandibular ramus
#56	Molar–premolar chord	D[44–45]	[68a]	Distance between alveolar processes in mid–points of 1st premolar and 2nd molar
#57	Maximum projective length of mandible	D[10–48]	[68(1)]	Maximum projective distance of mandible on sagittal plane
#58	Projective length of the corpus mandibulae	D[10–30]	[68]	Projective length from pogonion to gonion on sagittal plane
#59	Coronoid height	D[L(4,9)–46]	[70(1)]	Projective vertical length from the coronoid process to mandibular plane
#60	Greatest depth of mandibular notch	D[L(42,46)–47]	[70(3)]	Distance from the deepest point of mandibular notch to the line between koronion and highest point of condylar process
#61	Condyle–coronoid length	D[42–46]	[71(1)]	Distance from koronion to highest point of condylar process
Angle				
#62	Cranial base angle	A[L(2,29)–L(5,6)]	[37(2)]	Angle between nasion–basion line and FH plane
#63	Total profile angle	A[L(2,35)–L(5,6)]	[72]	Angle between nasion–prosthion line and FH plane
#64	Profile angle of nasal bones	A[L(2,37)–L(5,6)]	[75]	Angle between nasion–rhinion line and FH plane
#65	Alveolar profile angle	A[L(35,39)–L(5,6)]	[74]	Angle between nasospinale–prosthion line and FH plane
#66	Tooth angle	A[L(16,35)–L(5,6)]	[74(2)]	Angle between prosthion–cutting edge of upper central incisor line and FH plane
#67	Profile angle of nose	A[L(2,39)–L(5,6)]	[73]	Angle between nasion–nasospinale line and FH plane
#68	Inclination angle of nasal bridge	A[35–2–37]	[75(1)]	Angle formed by prosthion–nasion–rhinion
#69	Facial angle	A[L(2,35)–L(20,21)]	[72(2)']	Angle between nasion–prosthion line and occlusal plane
#70	Profile angle	A[2–35–49]	[72(4)]	Angle formed by nasion–prosthion–earhole central point
#71	Nasion angle (basion–prosthion)	A[35–2–29]	[72b]	Angle formed by basion–nasion–prosthion
#72	Prosthion angle (basion–nasion)	A[2–35–29]	[72(5)]	Angle formed by nasion–prosthion–basion
#73	Basion angle (nasion–prosthion)	A[2–29–35)]	[72c]	Angle formed by nasion–basion–prosthion
#74	Sagittal inclination angle of orbit	A[5–6–50]	[78]	Angle between FH plane and the line between the highest and lowest points of orbital margin
#75	Inclination angle of coronoid–condyle line	A[L(42,46)–L(14,15)]	[79(3)]	Angle between koronion–highest point of codylar process line and posterior tangent of ramus
#76	Symphyseal angle	A[38–4–9]	[79 (1a)]	Angle between infradentale–menton line and mandibular plane
#77	Mental angle	A[L(10,38)–L(4,9)]	[79c]	Angle between infradentale–pogonion line and mandibular plane

Appendix 2. (continued)

#	Measurements	Landmarks used*	Martin's #**	Definitions
#78	Menton–infradentale–pogonion angle	A[4–38–10]		Angle formed by menton–infradentale–pogonion
#79	Profile angle of mandible	A[L(10,38)–L(5,6)]	[79(1)]	Angle between infradentale–pogonion line and FH plane
Soft tissue				
Distance				
#80	Projected head length	D[51–52]	[1a–01]	Distance from grabella' to opisthokranion'
#81	Head length from nasion'	D[53–52]	[1d]	Distance from nasion' to opisthokranion'
#82	Chin to opisthokranion'	D[52–54]	[1–03]	Distance from supragnathion' to opisthocranion'
#83	Tragion' to back of head	D[55–54]	[2c]	Projective length from tragion' to opisthocranion' on sagittal plane
#84	Morphological facial height	D[53–56]	[18]	Distance from nasion' to menton'
#85	Sellion to menton'	D[57–56]	[18c]	Distance from sellion to menton'
#86	Glabella' to menton'	D[51–56]	[18–01]	Distance from grabella' to menton'
#87	Sellion to stomion	D[57–58]	[19b]	Distance from sellion to stomion
#88	Physiognomic upper facial height	D[53–58]	[19]	Distance from nasion' to stomion
#89	Nasal height	D[22–53]	[21]	Direct length from nasion' to subnasale'
#90	Nasal depth	Dx[22–59]	[22]	Projective anteroposterior length from pronasale to subnasale'
#91	Nasal length	D[53–59]	[23]	Distance from nasion' to pronasale
#92	Sellion–subnasale height	D[22–57]	[21c]	Distance from sellion to subnasale'
#93	Projective lower facial height	Dy[56–58]	[28c]	Projective length from stomion to menton'
#94	Mandible depth	D[30–56]	[28d]	Projective distance from menton' to gonion'
#95	Subnasale' to menton'	D[22–56]	[28(2)]	Distance from subnasale' to menton'
#96	Menton' t-radius	D[55–56]	[37–01]	Projective distance from tragion' to menton'
#97	Stomion t-radius	D[55–58]	[38a–01]	Projective distance from tragion' to stomion'
#98	Nasion' t-radius	D[55–53]	[95]	Projective distance from tragion' to nasion'
#99	Pronasale t-radius	D[55–59]	[39a–01]	Projective distance from tragion' to pronasale'
#100	Sellion t-radius	D[55–57]	[39b–01]	Projective distance from tragion' to sellion'
#101	Subnasale t-radius	D[55–22]	[39(1)–01]	Projective distance from tragion' to subnasale'
#102	Glabella' t-radius	D[55–51]	[40–01]	Projective distance from tragion' to glabella'

* D[a–b], distance between a and b; Dx[a–b] and Dy[a–b], distance between a and b in the direction horizontal and vertical to the FH plane, respectively; L(a,b), line formed by a and b; A[a–b–c] and A[L(a,b)–L(c,d)], angle formed by a–b–c and two lines, respectively. **See Knussmann (1988).

Appendix 3. The regional difference in the craniofacial measurements

#	Variables	Ryukyu		Mainland Japan		Difference *P* value	Significance **B-H method *$P < 0.05$
		Mean	SD	Mean	SD		
#1	S–N plane	69.07	3.46	69.79	3.00	0.39	
#2	Lower facial height	69.14	5.34	69.23	4.65	0.95	
#3	Middle facial height	54.12	3.83	58.34	2.55	6.8.E–06	**
#4	Full facial height	123.26	7.12	127.56	4.94	0.0089	*
#5	UM to palatal plane	25.30	2.75	24.40	1.70	0.13	
#6	LM to mandibular plane	36.93	2.23	36.82	2.08	0.85	
#7	Facial angle	86.05	4.56	85.87	4.36	0.87	
#8	Angle of convexity	5.21	7.14	6.47	5.87	0.46	
#9	A–B plane to facial plane angle	4.68	3.43	5.20	3.21	0.55	
#10	Mandibular plane angle	28.75	6.32	31.05	6.71	0.18	
#11	Y axis angle	63.77	4.09	64.45	4.15	0.53	
#12	Gonial angle	123.81	6.66	126.68	7.07	0.11	
#13	SNA angle	82.13	4.04	80.97	3.99	0.27	
#14	SNB angle	79.03	3.87	77.55	3.87	0.14	
#15	ANB angle	3.10	2.89	3.42	2.33	0.63	
#16	Interincisal angle	122.09	10.45	125.45	10.05	0.21	
#17	U1 to FH angle	115.03	7.03	113.55	7.35	0.43	
#18	U1 to SN angle	108.50	7.21	105.54	7.42	0.12	
#19	L1 to occlusal plane angle	67.98	7.75	71.15	7.49	0.11	
#20	L1 to mandibular plane angle	94.13	7.11	89.94	6.86	0.024	*
#21	Occlusal plane angle	10.91	4.64	12.14	4.78	0.31	
#22	Upper lip protusion	6.48	1.76	6.07	1.96	0.40	
#23	Lower lip protusion	6.50	2.49	6.16	2.71	0.61	
#24	Labiomental sulcus	4.43	1.24	4.45	1.08	0.94	
#25	Point A to subnasale	13.16	2.48	14.59	1.34	0.0077	*
#26	Incision superioris to upper lip	11.62	1.78	13.11	2.07	0.0043	**
#27	Incision inferioris to lower lip	14.10	1.80	14.75	1.99	0.19	
#28	Pogonion to pogonion’	12.18	2.48	12.24	2.76	0.92	
#29	Nasolabial angle	92.12	12.15	93.45	11.42	0.66	
#30	Z angle	69.37	10.19	67.41	10.64	0.47	
#31	Maximum cranial length	190.97	6.42	190.77	6.31	0.90	
#32	Nasion-opisthocranion length	186.63	6.26	187.37	5.96	0.64	
#33	Basion–nasion length	105.25	4.24	107.41	4.56	0.063	
#34	Basion–opisthocranion length	91.75	5.96	90.92	6.80	0.62	
#35	Nasion–supraglabellare chord	29.93	4.89	28.78	4.68	0.36	
#36	Glabellar projection	2.42	0.77	2.41	1.04	0.95	
#37	Supraorbitale–menton length	134.68	7.26	137.82	5.35	0.062	
#38	Supraorbitale–prosthion length	82.09	5.29	84.54	3.55	0.040	*
#39	Total facial height	125.17	7.32	129.27	4.82	0.013	*
#40	Upper facial height	72.96	4.63	76.23	2.85	0.0019	**
#41	Prosthion–menton height	56.04	4.23	56.16	3.71	0.91	
#42	Alveolar field height	19.76	3.03	18.57	2.09	0.081	
#43	Zygomatic field height	24.43	3.44	24.96	3.49	0.55	
#44	Basion–prosthion length	101.63	5.23	102.93	5.84	0.37	
#45	Basion–menton length	107.64	5.73	111.66	7.03	0.018	*
#46	Nasion–orbitale length	27.80	3.07	28.36	2.49	0.45	
#47	Nasion–nasospinale height	54.45	3.63	58.48	2.70	1.0.E–05	**
#48	Rhinion–nasospinale height	33.92	2.76	35.63	1.83	0.0068	**
#49	Length of nasal bones	24.72	3.41	29.09	2.87	1.6.E–06	**
#50	Orbitale–nasospinale height	26.30	3.04	29.97	2.54	4.8.E–06	**
#51	Height of mandibular symphysis	35.87	3.94	35.34	2.65	0.54	
#52	Height of mandibular body	29.97	2.61	29.93	2.34	0.96	

Appendix 3. (continued)

#	Variables	Ryukyu		Mainland Japan		Difference *P* value	Significance **B-H method *P* < 0.05
		Mean	SD	Mean	SD		
#53	Height of mandibular body (M2)	25.47	2.74	23.86	2.29	0.016	*
#54	Projective length of the ramus	59.56	5.25	60.36	4.71	0.54	
#55	Minimum breadth of ramus	29.66	3.39	31.48	3.49	0.045	*
#56	Molar–premolar chord	27.59	2.42	29.10	1.90	0.010	*
#57	Maximum projective length of mandible	109.76	5.80	113.52	5.82	0.015	*
#58	Projective length of the corpus mandibulae	77.71	4.93	78.50	6.45	0.60	
#59	Coronoid height	62.87	5.24	62.49	5.78	0.79	
#60	Greatest depth of mandibular notch	9.51	2.84	12.11	2.89	8.9.E–04	**
#61	Condyle-coronoid length	36.45	3.95	38.94	3.07	0.0086	*
#62	Cranial base angle	26.01	3.76	26.26	3.22	0.79	
#63	Total profile angle	87.23	4.64	88.09	3.32	0.41	
#64	Profile angle of nasal bones	68.06	7.65	64.57	5.70	0.051	
#65	Alveolar profile angle	70.77	6.53	73.93	6.47	0.065	
#66	Tooth angle	83.78	7.62	88.02	9.10	0.055	
#67	Profile angle of nose	93.14	5.76	92.55	3.26	0.63	
#68	Inclination angle of nasal bridge	19.18	5.51	23.51	5.23	0.0028	**
#69	Facial angle	76.37	3.57	75.95	3.08	0.63	
#70	Profile angle	64.90	3.83	63.84	2.27	0.20	
#71	Nasion angle (basion–prosthion)	66.75	3.52	65.65	3.29	0.21	
#72	Prosthion angle (basion–nasion)	71.99	2.59	71.87	2.39	0.86	
#73	Basion angle (nasion–prosthion)	41.26	2.74	42.47	2.44	0.074	
#74	Sagittal inclination angle of orbit	93.45	5.98	96.39	3.90	0.029	*
#75	Inclination angle of coronoid–condyle line	70.46	6.24	68.15	6.61	0.17	
#76	Symphyseal angle	91.14	6.21	86.47	5.34	0.0029	**
#77	Mental angle	74.48	7.35	72.90	5.91	0.36	
#78	Menton–infradentale–pogonion angle	34.28	6.92	38.77	5.14	0.0061	**
#79	Profile angle of mandible	76.77	9.46	76.05	9.27	0.77	
#80	Projected head length	203.17	6.31	203.46	6.60	0.86	
#81	Head length from nasion’	198.75	6.44	199.80	6.43	0.53	
#82	Chin to opisthokranion’	228.44	8.96	229.23	9.13	0.74	
#83	Tragion’ to back of head	109.07	7.13	109.62	5.99	0.74	
#84	Morphological facial height	134.80	7.83	138.65	4.97	0.027	*
#85	Sellion to menton’	128.09	7.57	130.40	5.02	0.17	
#86	Glabella’ to menton’	154.31	7.09	154.08	5.64	0.89	
#87	Sellion to stomion	77.77	4.99	79.92	3.96	0.070	
#88	Physiognomic upper facial height	84.25	5.06	88.31	3.71	8.3.E–04	**
#89	Nasal height	59.59	4.26	63.60	3.03	1.0.E–04	**
#90	Nasal depth	14.48	2.39	14.61	1.79	0.81	
#91	Nasal length	48.49	3.53	53.47	3.74	1.8.E–06	**
#92	Sellion–subnasale height	53.21	4.39	55.26	3.37	0.048	*
#93	Projective lower facial height	47.94	4.99	47.96	4.39	0.99	
#94	Mandible depth	74.06	5.06	76.41	6.27	0.11	
#95	Subnasale’ to menton’	79.91	5.14	80.69	4.20	0.52	
#96	Menton’ t-radius	121.13	7.54	124.36	7.36	0.10	
#97	Stomion t-radius	111.87	5.73	114.76	5.60	0.053	
#98	Nasion’ t-radius	100.49	5.53	100.76	4.61	0.84	
#99	Pronasale t-radius	116.08	4.76	118.79	5.61	0.049	*
#100	Sellion t-radius	97.26	4.81	98.29	4.50	0.40	
#101	Subnasale t-radius	104.49	4.57	107.43	5.30	0.025	*
#102	Glabella’ t-radius	111.39	6.24	109.42	3.95	0.15	

Appendix 4. Eigenvalues, contributions and loadings in PCA

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	19.3	14.3	10.4	7.5	7.0
Contribution (%)	18.9	14.0	10.2	7.4	6.9
Loading					
#1: S-N plane	0.549	–0.009	–0.087	0.456	–0.308
#2: Lower facial height	0.636	0.301	0.165	–0.517	–0.183
#3: Middle facial height	0.719	–0.215	–0.254	0.235	0.378
#4: Full facial height	0.920	0.103	–0.025	–0.257	0.086
#5: UM to palatal plane	0.554	0.129	0.120	–0.475	–0.244
#6: LM to mandibular plane	0.535	0.287	0.390	–0.182	–0.073
#7: Facial angle	–0.040	0.898	–0.032	–0.098	0.338
#8: Angle of convexity	0.063	–0.519	0.655	0.215	0.388
#9: A-B plane to facial plane angle	–0.007	–0.485	0.374	0.185	0.556
#10: Mandibular plane angle	0.282	–0.775	0.130	–0.180	–0.301
#11: Y axis angle	0.132	–0.857	0.061	–0.153	–0.246
#12: Gonial angle	0.286	–0.480	0.166	–0.206	–0.199
#13: SNA angle	–0.372	0.317	0.528	0.060	0.268
#14: SNB angle	–0.391	0.664	0.160	–0.047	–0.046
#15: ANB angle	0.012	–0.507	0.574	0.162	0.482
#16: Interincisal angle	–0.036	0.044	–0.709	–0.203	0.288
#17: U1 to FH angle	0.066	0.687	0.263	–0.021	–0.185
#18: U1 to SN angle	–0.140	0.519	0.268	–0.017	–0.401
#19: L1 to occlusal plane angle	–0.009	0.216	–0.792	–0.261	0.104
#20: L1 to mandibular plane angle	–0.270	–0.040	0.631	0.474	0.046
#21: Occlusale plane angle	–0.037	–0.773	–0.141	0.048	–0.174
#22: Upper lip protusion	0.176	–0.229	0.461	0.359	–0.049
#23: Lower lip protusion	0.182	–0.275	0.686	0.088	–0.050
#24: Labiomental sulcus	–0.072	0.087	–0.395	0.473	0.087
#25: Point A to subnasale	–0.055	0.031	–0.275	0.289	0.062
#26: Incision superioris to upper lip	–0.024	0.029	–0.568	0.349	0.314
#27: Incision inferioris to lower lip	–0.050	–0.024	–0.393	0.271	0.249
#28: Pogonion to pogonion’	0.125	0.282	–0.072	0.001	–0.039
#29: Nasolabial angle	0.032	–0.278	0.319	0.000	0.122
#30: Z angle	–0.047	0.753	–0.456	–0.237	0.014
#31: Maximum cranial length	0.482	–0.039	0.125	0.146	–0.423
#32: Nasion-opisthocranion length	0.552	–0.067	0.121	0.215	–0.376
#33: Basion-nasion length	0.578	0.178	–0.046	0.456	–0.181
#34: Basion-opisthocranion length	0.229	–0.015	0.170	–0.233	–0.029
#35: Nasion-supraglabellare chord	–0.210	0.016	0.221	–0.398	–0.110
#36: Glabellar projection	–0.044	0.079	0.169	–0.273	–0.050
#37: Supraorbitale-menton length	0.804	–0.130	0.105	–0.349	–0.002
#38: Supraorbitale-prosthion length	0.569	–0.374	0.021	–0.161	0.216
#39: Total facial height	0.940	–0.068	–0.013	–0.251	0.016
#40: Upper facial height	0.821	–0.313	–0.141	–0.031	0.269
#41: Prosthion-menton height	0.704	0.077	0.456	–0.339	–0.211
#42: Alveolar field height	0.256	–0.170	0.200	–0.374	–0.245
#43: Zygomatic field height	0.259	–0.099	0.212	0.081	0.037
#44: Basion-prosthion length	0.346	0.333	0.356	0.561	–0.039
#45: Basion-menton length	0.483	0.563	–0.182	0.228	–0.048
#46: Nasion-orbitale length	0.491	0.195	–0.169	–0.042	0.284
#47: Nasion-nasospinale height	0.715	–0.172	–0.297	0.216	0.346
#48: Rhinion-nasospinale height	0.428	–0.007	–0.159	0.184	0.213
#49: Length of nasal bones	0.489	–0.216	–0.345	–0.004	0.397
#50: Orbitale-nasospinale height	0.407	–0.350	–0.164	0.303	0.203
#51: Height of mandibular symphysis	0.571	0.089	0.403	–0.399	–0.260

Appendix 4. (continued)

	PC1	PC2	PC3	PC4	PC5
#52: Height of mandibular body	0.575	0.221	0.473	–0.228	–0.218
#53: Height of mandibular body (M2)	0.252	0.403	0.260	–0.082	–0.011
#54: Projective length of the ramus	0.309	0.586	–0.067	–0.176	0.260
#55: Minimum breadth of ramus	0.177	0.386	–0.020	0.268	–0.007
#56: Molar-premolar chord	0.515	0.039	0.152	0.013	0.260
#57: Maximum projective length of mandible	0.658	0.450	–0.118	–0.033	–0.124
#58: Projective length of the corpus mandibulae	0.322	0.617	–0.302	0.225	–0.115
#59: Coronoid height	0.213	0.507	–0.097	–0.159	0.194
#60: Greatest depth of mandibular notch	0.315	0.177	–0.251	–0.040	0.198
#61: Condyle-coronoid length	0.119	0.122	0.083	0.143	0.145
#62: Cranial base angle	0.175	0.378	0.082	–0.361	0.483
#63: Total profile angle	0.062	–0.663	–0.541	–0.014	–0.425
#64: Profile angle of nasal bones	0.059	–0.351	–0.184	0.125	–0.594
#65: Alveolar profile angle	0.093	–0.620	–0.292	–0.039	0.207
#66: Tooth angle	–0.001	–0.661	–0.393	0.061	0.232
#67: Profile angle of nose	0.017	–0.477	–0.434	–0.074	–0.658
#68: Inclination angle of nasal bridge	–0.028	–0.041	–0.156	–0.160	0.418
#69: Facial angle	0.125	0.315	–0.445	–0.087	–0.261
#70: Profile angle	–0.100	–0.351	–0.490	0.158	–0.619
#71: Nasion angle (basion-prosthion)	–0.250	0.395	0.553	0.382	0.009
#72: Prosthion angle (basion-nasion)	–0.076	–0.024	–0.506	–0.069	–0.385
#73: Basion angle (nasion-prosthion)	0.395	–0.490	–0.242	–0.430	0.349
#74: Sagittal inclination angle of orbit	0.301	–0.299	–0.116	0.251	–0.135
#75: Inclination angle of coronoid-condyle line	–0.090	0.098	–0.026	–0.064	0.001
#76: Symphyseal angle	–0.223	0.171	0.609	0.277	0.094
#77: Mental angle	–0.097	–0.188	0.729	0.377	0.114
#78: Menton-infradentale-pogonion angle	0.089	–0.304	0.053	0.085	0.178
#79: Profile angle of mandible	–0.130	0.682	–0.614	–0.143	0.131
#80: Projected head length	0.555	0.025	0.164	0.185	–0.403
#81: Head length from nasion’	0.619	0.017	0.134	0.230	–0.294
#82: Chin to opisthokranion’	0.460	0.527	–0.001	–0.042	–0.005
#83: Tragion’ to back of head	0.151	–0.239	0.264	–0.195	0.006
#84: Morphological facial height	0.913	–0.088	0.006	–0.227	0.011
#85: Sellion to menton’	0.816	–0.106	0.152	–0.301	0.082
#86: Glabella’ to menton’	0.590	–0.102	0.121	–0.229	–0.234
#87: Sellion to stomion	0.612	–0.357	0.091	–0.019	0.341
#88: Physiognomic upper facial height	0.790	–0.346	–0.096	0.060	0.247
#89: Nasal height	0.770	–0.244	–0.265	0.143	0.242
#90: Nasal depth	0.295	–0.248	–0.320	–0.161	–0.148
#91: Nasal length	0.543	–0.239	–0.255	0.262	0.352
#92: Sellion-subnasale height	0.538	–0.245	–0.058	0.063	0.346
#93: Projective lower facial height	0.477	0.608	0.121	–0.426	–0.068
#94: Mandible depth	0.365	0.441	–0.290	0.215	–0.076
#95: Subnasale’ to menton’	0.654	–0.080	0.409	–0.315	–0.081
#96: Menton’ t-radius	0.604	0.708	–0.082	–0.016	0.084
#97: Stomion t-radius	0.572	0.520	0.152	0.494	0.057
#98: Nasion’ t-radius	0.552	0.021	–0.201	0.510	–0.385
#99: Pronasale t-radius	0.564	0.281	–0.057	0.613	0.004
#100: Sellion t-radius	0.586	–0.001	–0.085	0.519	–0.299
#101: Subnasale t-radius	0.528	0.354	0.043	0.694	0.057
#102: Glabella’ t-radius	0.256	–0.050	–0.108	0.415	–0.577