RESULTS

Description and reconstruction of shapes

The description of ischial callosity shape as a series of landmark and its reconstruction is given in Appendix 2. Based on visual judgment, reconstructions were successfully represent original shape (data not shown).

Shape variation and its pattern

Variation in each shape was described as affine and non-affine deformations (Appendix 3 and 4). Average shapes of Sulawesi macaques OTU, M. nemestrina, and M. fascicularis are shown in Figure 5; these averages were used as reference for deformation analysis.

![Figure 5: Average shape of each OTU, a) Sulawesi macaques, b) M. nemestrina, and c) M. fascicularis. Dots are their landmarks.](image)

The individual position in the affine shape space of each OTU is shown in Figure 6a for Sulawesi macaques, Figure 7a for M. nemestrina, and Figure 8a for M. fascicularis. Ischial callosity variation pattern in a particular direction were included in these figures in the form of deformation grids. The parallel lines in these grids remain parallel, representing global change. For non-affine components, the variation was summarized by relative warps. The individual position in the first two relative warps (RW) is shown in Figure 6b for Sulawesi macaques, Figure 7b for M. nemestrina, and Figure 8b for M. fascicularis. Ischial callosity variation pattern in a particular direction were also included in these figures in the form of deformation grids. The unequally bending lines in the grids represent localized change.
Figure 6 Position of each individual (dot) of Sulawesi macaques in (a) affine shape space, and (b) RW 1 (carries 44.38% of variation) and RW 2 (19.18%) which represent non-affine change. The origin (0,0) is their average shape (Figure 5a).
Figure 7  Position of each individual (dot) of *M. nemestrina* in (a) affine shape space, and (b) RW 1 (55.82%) and RW 2 (17.10%) which represent non-affine change. The origin (0,0) is their average shape (Figure 5b).
Figure 8 Position of each individual (dot) of *M. fascicularis* in (a) affine shape space, and (b) RW 1 (49.18%) and RW 2 (21.94%) which represent non-affine change. The origin (0,0) is their average shape (Figure 5c).
All three OTU showed a concentric scatter of individuals around the origin in both affine and non-affine shape space. These results indicate that the variation within each OTU was normally distributed from its average shape. But the range of this distribution along the x- and y-axis were not the same for each OTU.

Sulawesi macaques showed significance deformation of its ischial callosity in the affine shape space compared to M. nemestrina. This indicated that the size and orientation of callosity in M. nemestrina was almost the same among all members. Their ischial callosity variation, thus, will be largely contributed by its non-affine components. Significance deformation in affine shape space was also showed by M. fascicularis. In the x-axis, shape was varied due to orientation and difference in the length of long axis of the ischial callosity. Meanwhile, its y-axis represented variation in shape due to difference in the ratio of axes in the ischial callosity (oval to circular). The magnitude of deformation seemed to be equal in Sulawesi macaques, while M. fascicularis showed greater magnitude of deformation in its x-axis.

The variation of non-affine components within Sulawesi macaques was greater than within either M. fascicularis or M. nemestrina. This was showed by relatively small cumulative value of its first two relative warps (63.55%), compared to M. fascicularis (71.12%) and M. nemestrina (72.92%). This result was predictable because the Sulawesi macaques OTU was composed of seven different species from many populations, while the M. fascicularis OTU was only composed of two populations (Pangandaran and Primate Research Center), and the M. nemestrina OTU only from a single population (Primate Research Center).

In the Sulawesi macaques, non-affine shape difference was largely contributed by the combination of a wide dorsal side with a small ventral side (RW 1 = 44.38%). The bending of the callosity was the next factor that influenced non-affine shape (RW 2 = 19.18%). The inward bending showed greater magnitude (two times) than the outward bending, although the numbers of individuals with inward or outward bending were almost the same.

The biggest non-affine component of M. nemestrina was responsible for the deformation of the outer part of ischial callosity (RW 1 = 55.82%). The RW 2
(17.10%) explained the deformation of the dorsal part of the callosity. This latter component made the callosity of *M. nemestrina* vary from oval to triangular.

*M. fascicularis* had the biggest contribution of non-affine shape difference from the deformation of inner and outer parts of the callosity (RW1 = 49.18%). Deformation of dorsal and ventral parts were explained by RW 2 (21.94%) and made the callosity vary from circle to oval.

The variation in each species of Sulawesi macaques was summarized by their relative warps (RW, see Table 3). The total number of RW needed to represent all variations can be used to determine the range of variation. A broader range of variation will produce more RW than a narrower one. But, it is obvious that the total number of RW produced in each species is highly correlated with the number of sample in that species (see Table 2). Therefore, the small amount of RW in *M. nigrescens* and *M. brunnescens* do not necessarily reflect a narrower range of variation in these species; and vice versa for southern species (except *M. brunnescens*).

<table>
<thead>
<tr>
<th>Species</th>
<th>RW 1 (%)</th>
<th>RW 2 (%)</th>
<th>RW 3 (%)</th>
<th>RW 4 (%)</th>
<th>RW 5 (%)</th>
<th>RW 6 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. nigra</em> (18 RW)</td>
<td>58.42</td>
<td>19.08</td>
<td>8.47</td>
<td>4.80</td>
<td>2.45</td>
<td>2.02</td>
</tr>
<tr>
<td><em>M. nigrescens</em> (5 RW)</td>
<td>60.39</td>
<td>19.73</td>
<td>15.86</td>
<td>2.74</td>
<td>1.27</td>
<td>-</td>
</tr>
<tr>
<td><em>M. hecki</em> (13 RW)</td>
<td>50.55</td>
<td>17.20</td>
<td>10.53</td>
<td>9.03</td>
<td>3.27</td>
<td>2.93</td>
</tr>
<tr>
<td><em>M. tonkeana</em> (23 RW)</td>
<td>58.42</td>
<td>15.90</td>
<td>10.02</td>
<td>4.53</td>
<td>3.35</td>
<td>2.55</td>
</tr>
<tr>
<td><em>M. maurus</em> (22 RW)</td>
<td>48.27</td>
<td>19.72</td>
<td>9.56</td>
<td>6.67</td>
<td>4.76</td>
<td>4.13</td>
</tr>
<tr>
<td><em>M. ochreata</em> (26 RW)</td>
<td>58.27</td>
<td>15.42</td>
<td>7.38</td>
<td>5.97</td>
<td>4.04</td>
<td>2.52</td>
</tr>
<tr>
<td><em>M. brunnescens</em> (6 RW)</td>
<td>78.55</td>
<td>12.24</td>
<td>5.18</td>
<td>2.43</td>
<td>0.93</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Relationship between shapes

The consensus images for each species of Sulawesi macaques are shown in Figure 9. Each image represents the generalized shape of its species. Based on visual judgment, these images could be separated into three types. The first is the oval without bending, possessed by *M. maurus* and *M. tonkeana*; the second is the oval with outward bending of *M. ochreata* and *M. brunnescens*; the last, possessed by all northern species (*M. hecki*, *M. nigrescens*, and *M. nigra*), is the inward bending, with an oval shape in *M. nigrescens* and reniform shape in *M. hecki* and *M. nigra*. The bending of callosity (explained by RW 2 in Figure 6b) is obviously an important factor to influence above type’s separation of callosity in Sulawesi macaques.

A neighbour-joining tree of Sulawesi macaques, *M. nemestrina*, and *M. fascicularis* is shown in Figure 10. This tree was generated from 8 RW (carries 100% of variation), computed based on average shape of each species. The earlier separation by visual judgment into three types is supported by this cluster analysis. The clustering was congruent with each of determined types of Sulawesi macaques’ ischial callosity (cf. Figure 9). Clusters that contained members from both Sulawesi (that is *M. maurus* and *M. tonkeana*), and from *M. nemestrina* or *M. fascicularis* were regarded as having the generalized shape of callosity. Other clusters were regarded as having derived shape, unique to that several species. *M. nemestrina* seems to have a unique triangular ischial callosity form and its position is closer to central and southern species than to three northern species. The three northern Sulawesi species are well separated due to their unique inward bending callosity, with a close relationship between *M. hecki* and *M. nigra*. The central Sulawesi species, *M. tonkeana*, shows great affinity with *M. fascicularis*. The southern Sulawesi species form a separate cluster, with close relationship between *M. ochreata* and *M. brunnescens*. These two latter species have an outward bending type of callosity.
Figure 9 Consensus images of ischial callosity from seven species of Sulawesi macaques (arranged according to their type, legend as Figure 1).
Figure 10 Neighbour-joining tree of Sulawesi macaques and 2 surrounding species which show relationship of shape based on 8 RW (carries 100% of variation). These RW were computed based on each species’ average shape. nm = \textit{M. nemestrina}, fc = \textit{M. fascicularis}, legend for Sulawesi macaques as Figure 1.
DISCUSSION

Quantitative methods

The present quantitative methods, Generalized Procrustes Analysis and Thin-plate Spline, open interesting perspectives for the analysis of shape of ischial callosity. It provides objective reconstruction of callosity shape and retains its morphological complexity. Individual variation can be compared and an average shape of determined group, such as species, can be generated. Decomposition of shape into affine and non-affine components revealed several suggestions about variation of callosity’s shape. Later, these components can be used to determine relationship among shape under study. The mathematical formulae also allow the callosity shape to be reproduced precisely without even prior knowledge of the shape before. All of these properties were lack in the previous ischial callosity shape analysis that employed verbal methods.

Shape variation and its pattern

Each species analyzed in this research showed a different kind of variation in its ischial callosity. In intra-species analysis, when all Sulawesi macaques were considered as a one OTU, they showed the lowest cumulative value of the first two relative warps (RW). The callosity variation in Sulawesi macaques is therefore obviously broader compared to M. nemestrina and M. fascicularis.

The biggest contribution to the variation of ischial callosity shape in Sulawesi macaques is from the differences in the dorsal part and the direction of bending (Figure 6b). Differences in the dorsal part were clearly a common factor of variation in all Sulawesi macaques. The bending of the callosity, on the other hand, was observed only in the northern and southeastern species. Figure 11 shows these conditions. Although the range of variation in each species mostly overlapped, there are several species that shows a non-overlapped range of variation. These species are M. nigra, M. nigrescens, M. hecki in the one hand, versus M. ochreata, and M. brunnescens in the other hand. All have a bending callosity but with reverse direction. Thus, there are three types of callosity determined in this research: the oval without bending (M. tonkeana and M.
maurus), the oval with outward bending (M. ochreata and M. brunnescens), and the oval and reniform shape with inward bending (M. hecki, M. nigrescens, and M. nigra). These types are congruent with the present geography of Sulawesi macaques and supported by cluster analysis that included all RW (Figure 1 and 10).

Fooden (1969) described the shape of the callosity in M. tonkeana and M. maurus as suboval. Suryobroto et al. (unpubl. data) expanded the shape range of these two species from suboval to oblong along with the southeastern species and M. nigrescens in one of their six ischial callosities descriptions (Table 1). These two species have a broad range of variation as shown by their number of RW (Table 3). I consider suboval of Fooden (1969) and suboval to oblong of Suryobroto et al. (unpubl. data) as oval, with the opportunity to distort and so resulting in this broad variation. As Fooden (1969) suggested that the suboval (or oval in this research) is the generalized form of callosity, then these two macaques would be the best candidates for species closest to the ancestral form of callosity.

Previous work by Fooden (1969) and Albrecht (1978) also proposed M. tonkeana and M. maurus, respectively, as most resembling the ancestral type.

Figure 11 Range of shape variation in each species. Legend as Figure 1 and 10.
In the southeastern peninsula, *M. ochreata* and *M. brunnescens* have the same type of ischial callosity, which is oval shape with outward bending. The close relationship of these two species is widely recognized. Several workers even consider *M. brunnescens* as a subspecies of *M. ochreata* (Groves 1980, 1997, 2000, Shoshani *et al.* 1996, Brandon-Jones 2004). Fooden (1969) described the ischial callosity shape of *M. ochreata* as suboval and *M. brunnescens* as suboval to subreniform, while Suryobroto *et al.* (unpubl. data) lumped them in the same group with *M. tonkeana*, *M. maurus*, and *M. nigrescens* as suboval to oblong (Table 1). As I proposed earlier for *M. tonkeana* and *M. maurus*, the suboval shape of ischial callosity in *M. ochreata* and *M. brunnescens* can be also considered as oval shape with some kind of distortion. The oval shape of *M. tonkeana* and *M. maurus* can be considered as generalized, but the outward bending of the other two species is unlikely to be. Therefore, although Watanabe *et al.* (1985) have proposed *M. ochreata* as the most generalized form of all Sulawesi macaques, this bending characteristic argues against it. Dr. B. Suryobroto (pers. comm.) has agreed that his 1985 proposal with Dr. T. Watanabe and Dr. Y. Hamada is probably no longer appropriate. The variations among members in these 2 species are not the same (see Table 3). *M. ochreata* has the greatest number of RW (26), while *M. brunnescens* has the second lowest number of RW (6). Ischial callosity variation in *M. ochreata* is, therefore, much greater than in *M. brunnescens*; the lower variability of the latter could be due to their restriction to Muna and Butung and low number of samples available in this research.

Fooden (1969) indicated that *M. brunnescens* has a non-suboval form of ischial callosity. Suryobroto *et al.* (unpubl. data) also offered two additional types of callosity that the four species mentioned above could have. Both recognized subreniform (even to reniform by latter workers) shape of ischial callosity for them (Table 1). Again, I prefer to lump all these variations as simple oval shape. I think that these workers’ descriptions were influenced by the variably dorsal part and the outward bending of the callosity to consider its shape as subreniform or reniform.
The three northern species (M. hecki, M. nigrescens, and M. nigra) are clearly separated from the rest of Sulawesi macaques by their inward bending callosity. Although they have something in common, these three species show different proportions of dorsal and ventral parts. M. hecki and M. nigrescens have almost equal size of dorsal and ventral ischial callosity, while M. nigra has a bigger dorsal part. Suryobroto et al. (unpubl. data) gave the same descriptions for M. hecki and M. nigra, although they refer to dorsal as upper and ventral as lower lobes. The callosities of these two species also possess a unique characteristic that this research could not capture: their dorsal and ventral part is divided by a transverse furrow (Fooden 1969, Suryobroto et al. unpubl. data). For M. nigrescens, the ischial callosity is oval shape, different from the reniform shape of the remaining two northern species, such that Suryobroto et al. (unpubl. data) combined M. nigrescens with the non-northern species types, and Fooden (1969) have a range from suboval to subreniform shape for M. nigrescens (Table 1). I have to note again that these workers might have been influenced by the bending of the callosity. M. nigra and M. nigrescens also have convex callosity, while the other Sulawesi macaques are rather flat (Groves pers. comm.). This characteristic, like the transverse furrow, was not captured in the analysis. M. nigrescens also have the lowest number of RW among the Sulawesi macaques; the cause is probably the same as with M. brunnescens, although the isolated population explanation is rather unlikely. Groves (1980) and Watanabe & Matsumura (1991) have found possible hybrids between M. nigrescens and M. nigra in the northern peninsula. M. hecki and M. nigra compared to central and southern species have fewer RW indicating their narrower range of shape variation.

The M. nemestrina variation is the lowest in this research, perhaps because they all come from single population, the breeding colony in the Primate Research Center at Bogor Agricultural University, and all have a Sumatra origin. The proportion of affine components (size, orientation, and location) in this species ischial callosity variation is small (Figure 7a). The variation is largely due to non-affine components, varying from oval to triangular (Figure 7b). As their average shape have shown (Figure 5b), I prefer the description as triangular. Suryobroto et
al. (unpubl. data) described the callosity shape of this species as suboval, whereas I think that the distortion from the oval shape is more significant than this.

Only poor descriptions have hitherto been available for the *M. fascicularis* ischial callosity shape. In this research, there were two populations sampled, at Pangandaran, and at the Primate Research Center at Bogor Agricultural University, a colony which originated from Tinjil island, and this restricted origin may explain their narrow variation compared to the Sulawesi macaques. The shape variation is in both affine and non-affine components; the affine component influenced mostly by the length of long axis, making them vary from circle to oval (Figure 8a), while distortion from the oval shape in different parts of the callosities marked their non-affine deformation (Figure 8b). Despite this variation, I can state that the ischial callosity shape of *M. fascicularis* is generally oval. This is strongly implied by its average shape (Figure 5c).

**Relationship between shapes**

The ancestors of Sulawesi macaques were postulated to resemble *M. nemestrina* (Fooden 1969). DNA evidence supports Fooden’s hypothesis (Evans et al. 1999, 2003a, 2003b, Deinard & Smith 2001, Tosi et al. 2000, and Morales & Melnick 1998). Idea of a *M. fascicularis* origin for Sulawesi macaques was not corroborated by latter research; also this species is believed to have dispersed into Indonesia after *M. nemestrina* (Fooden 1969, 1976, Eudey 1980, Brandon-Jones 1996). The close relationship in the shape of the ischial callosity between *M. tonkeana* and *M. fascicularis* in this research does not reflect a close relationship as a species (Figure 10) but, rather, as Fooden (1969) proposed, the generalized state.

The oval shape and unbending callosities of *M. tonkeana* and *M. maurus* reflect a retention of the ancestral form, while *M. ochreata* and *M. brunnescens* developed an outward bending form and *M. nemestrina* developed a triangular form from this oval shape. In northern peninsula, I think that *M. hecki* and *M. nigra* in the early stages of their existence must have had an oval shape callosity resembled *M. tonkeana* but with inward bending, and that later this changed to
reniform shape with stronger inward bending (Figure 9) and a transverse furrow. *M. nigrescens* seems to retain more oval shape than those two.

After the initial colonization and speciation of Sulawesi macaques there were probably several minor events. The range of these species must have been in contact at the past; even today, there are several contact zones between them (Groves 1980, Supriatna et al. 1990, 1992, Watanabe & Matsumura 1991, Watanabe et al. 1991a, 1991b, Bynum 1995, Froehlich & Supriatna 1996). Gene flow from neighboring species was surely pervasive, which is why we can see a mixture of generalized and derived character states in each species of the Sulawesi macaques, including its ischial callosity (Figure 11).