

# Geographical Variation in the Clouded Leopard, *Neofelis nebulosa*, Reveals Two Species

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## Summary

The clouded leopard, *Neofelis nebulosa*, is an endangered semiarboreal felid with a wide distribution in tropical forests of southern and southeast Asia, including the islands of Sumatra and Borneo in the Indonesian archipelago [1]. In common with many larger animal species, it displays morphological variation within its wide geographical range and is currently regarded as comprising of up to four subspecies [2–4]. It is widely recognized that taxonomic designation has a major impact on conservation planning and action [5–8]. Given that the last taxonomic revision was made over 50 years ago [2], a more detailed examination of geographical variation is needed. We describe here the results of a morphometric analysis of the pelages of 57 clouded leopards sampled throughout the species' range. We conclude that there are two distinct morphological groups, which differ primarily in the size of their cloud markings. These results are supported by a recent genetic analysis [9]. On that basis, we give diagnoses for the distinction of two species, one in mainland Asia (*N. nebulosa*) and the other in Indonesia (*N. diardi*). The implications for conservation that arise from this new taxonomic arrangement are discussed.

## Results and Discussion

The taxonomic history of *Neofelis nebulosa* is complex (see the [Supplemental Data](#) available with this article

online). Currently, on the basis of a handful of specimens, four regional groupings are recognized as subspecies of *Neofelis nebulosa* [2–4] (Table 1 and Figure 1). This classification has remained unchanged for more than 50 years, without any review of geographical variation in morphology. However, with progressive fragmentation and destruction of habitat and hunting for use in traditional Chinese medicine and for the fur trade, the clouded leopard is increasingly endangered [1]. It is regarded as Vulnerable by the World Conservation Union and is currently on CITES (Convention on International Trade in Endangered Species) Appendix 1, which bans completely international trade [1].

In recent years, there have been comprehensive reviews of geographical variation in endangered mammals and birds, and these reviews have been made possible by larger samples and the application of new techniques, more sophisticated statistical analyses, and changing species concepts. Indeed, taxonomy and species concepts have changed considerably in light of the fact that many of these taxa were first described in the nineteenth century. From original species and subspecies descriptions based on single or few specimens from extremes of the geographical range, modern taxonomic revision requires extensive samples from throughout the species' range [8, 10]. Developments in primate taxonomy [8] serve as a useful example. Recent taxonomic reviews have been influenced by the phylogenetic species concept (PSC [11]), in the commonest version of which species are defined to be groups of individuals that share at least one uniquely derived character. This has resulted in a significant increase in the number of recognized species, and the number of endangered species, the advantages and disadvantages of which have been the subject of some debate [6].

Given the paucity of samples on which the taxonomy of the clouded leopard has been based, its current rarity, and modern trends in taxonomic analysis, a review of the geographical variation in the clouded leopard is timely, particularly if it can help to inform those planning future conservation strategies both in the wild and in captivity [5]. In this paper, we examine the pelage characters of the endangered clouded leopard throughout its geographical range by examining all available specimens in major museums in Europe and North America (Tables 1 and 2 and the [Supplemental Data](#) available with this article online). Ten characters (Table 3) were scored from 55 museum skins as well as a further two skins from a private collection.

We found that 27 of the 45 possible associations between characters, as measured by Kendall's  $\tau$ , were nonsignificant, eight were significant only at the 0.05 level, two were significant only at the 0.01 level, and eight were significant at the 0.001 level (Table S1). In summary, clouded leopards with large clouds tend to have fewer, often faint, spots within the cloud markings, and they are lighter in color, with a tendency toward

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Table 1. Current Subspecific Taxonomy of the Clouded Leopard, *Neofelis nebulosa*

Subspecies	Geographical Distribution
<i>Neofelis nebulosa nebulosa</i> (Griffith, 1821) [15]	SE Asia, China
<i>Neofelis nebulosa diardi</i> (Cuvier, 1823) [18]	Sumatra, Borneo
<i>Neofelis nebulosa macrosceloides</i> (Hodgson, 1853) [16]	India, Nepal, Sikkim, Bhutan
<i>Neofelis nebulosa brachyura</i> (Swinhoe, 1863) [17]	Formosa (Taiwan)

tawny-colored fur and a partial double dorsal stripe, whereas clouded leopards with small clouds tend to have many distinct spots within the cloud markings, greyer fur, and a double dorsal stripe.

Ordination by means of nonmetric multidimensional scaling indicates a clear separation into two groups, primarily on the first axis (Figure 2). This distinguishes individuals from Borneo and Sumatra (and the Batu islands) from the rest, which will be subsequently referred to as “mainland,” even though they include individuals from Taiwan and Palawan. Clouded leopards from Borneo and Sumatra all have small cloud markings, in contrast to the large clouds of mainland animals, and therefore it is not surprising that they group together. Examination of the association between the score on the first axis and individual characters suggest that a high score is associated with large cloud size, a low number of cloud spots,

Table 2. Samples of Clouded-Leopard Pelages Examined in This Study in Accordance to Collecting Locality and Putative Subspecies

ID	Locality	Subspecies	n
1	Borneo	<i>diardi</i>	8
2	Sumatra	<i>diardi</i>	14
2	Batu Islands	<i>diardi</i>	2
3	India, Nepal	<i>macrosceloides</i>	10
4	China, SE Asia	<i>nebulosa</i>	19
5	Taiwan	<i>brachyura</i>	3
6	Palawan	Unassigned	1

Two specimens from the Batu Islands were included in the Sumatran sample owing to geographical proximity. Clouded leopards are unknown from Palawan and hence cannot be assigned to a known subspecies.

and a tawny pelage that is not gray (Kendall's  $\tau$ : 0.70,  $-0.56$ ,  $0.37$ , and  $-0.42$ , respectively; see Table S2).

The same analysis was carried out on the other nine pelage characters, excluding cloud size (Figure S1). In this case, there is no longer a clear separation into two groups. However, there is still evidence of some separation of individuals from Borneo and Sumatra from the others. The score of each individual on the first axis differs significantly between Indonesian individuals (Borneo and Sumatra) and those from the mainland ( $p \approx 0.003$ ).

Discriminant analysis shows that the pelage characters other than cloud size can be used for discriminating

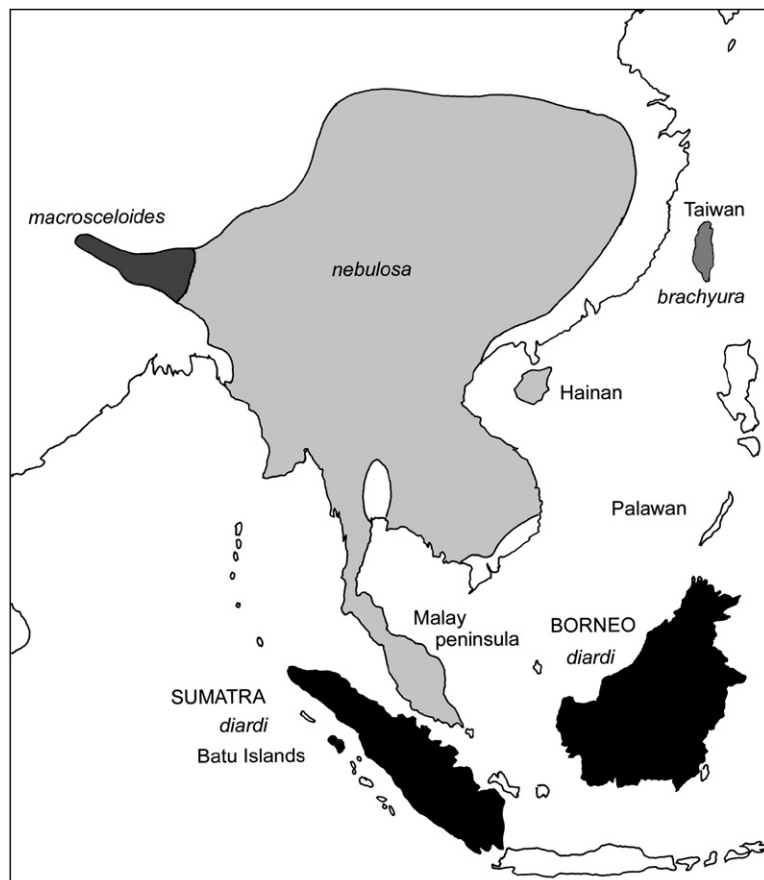


Figure 1. Geographical Distribution of Putative Clouded-Leopard Subspecies

This figure shows the geographical distribution of the clouded leopard and the location of the original four subspecies.

Table 3. Pelage Characters of Clouded Leopards Used in This Study

Characters	Scoring of Characters
Cloud size (CSI)	The cloud spots were scored on a two-point scale from “small” to “large.” “Large” almost rectangular clouds extended the full depth of the flank and sloped backwards from the dorsal midline in the forepart of the body, whereas “small” irregular clouds formed two or more rows in the same region arranged vertically from the dorsal midline. In about 20% of large-cloud specimens, one or occasionally two clouds have broken into two clouds, but this does not affect their categorization. No intermediates were recorded.
Number of cloud spots (CSP)	The degree of spotting was scored initially on a three-point scale from “few/none” (<10 spots/cloud) through “medium” (11–15 spots) to “many” (16+). Scoring revealed intermediate cases between these categories where numbers of spots varied between clouds in the same individual, and so these were given intermediate scores between the two commonest categories, extending this to a six-point scale.
Pelage lightness (LGT)	The pelage darkness was scored initially on a three-point scale from “light/pale” to “medium” and “dark” that increased from “dark” to “pale” or “light.” In some cases, it was difficult to assign a category and thus intermediate scores were given between categories, extending this to a five-point scale.
Pelage brightness (BRI)	The brightness of the pelage was scored initially on a three-point scale from “low” through “medium” to “high.” In some cases, it was difficult to assign a category and thus intermediate scores were given between categories, extending this to a five-point scale.
Dorsal stripe (DST)	The degree of dorsal striping was scored on a three-point scale from “single” to “double.” The intermediate category consisted of partially double stripes.
Neck stripes (NST)	The degree of neck striping was scored initially on a five-point scale based on the thickness of the two outer- and two inner-neck stripes, ranging from “two outer stripes thick, vestigial inner stripes” to “four stripes, all thick.” Intermediate scores in ascending score order were given for “one outer thick, other three thin,” “two outer thick, two inner thin,” and “two outer thick, two inner moderate.” Neck stripes clearly fell into five subcategories.
Shoulder patterning (SHP)	The pattern of two stripes at the shoulder was scored initially on a three-point scale from “narrow” through “intermediate” to “wide.” In some cases, it was difficult to assign a category and thus intermediate scores were given between categories, and a further highest-scoring category “fused” was recorded, extending this to a five-point scale.
Coloration scored on three scales: yellow (YEL), tawny (TAW), and gray (GRY)	Each scale was defined as a separate character, which could either be “absent” or “present.” This was a visual assessment and took account of pelages that had a combination of evident colors.

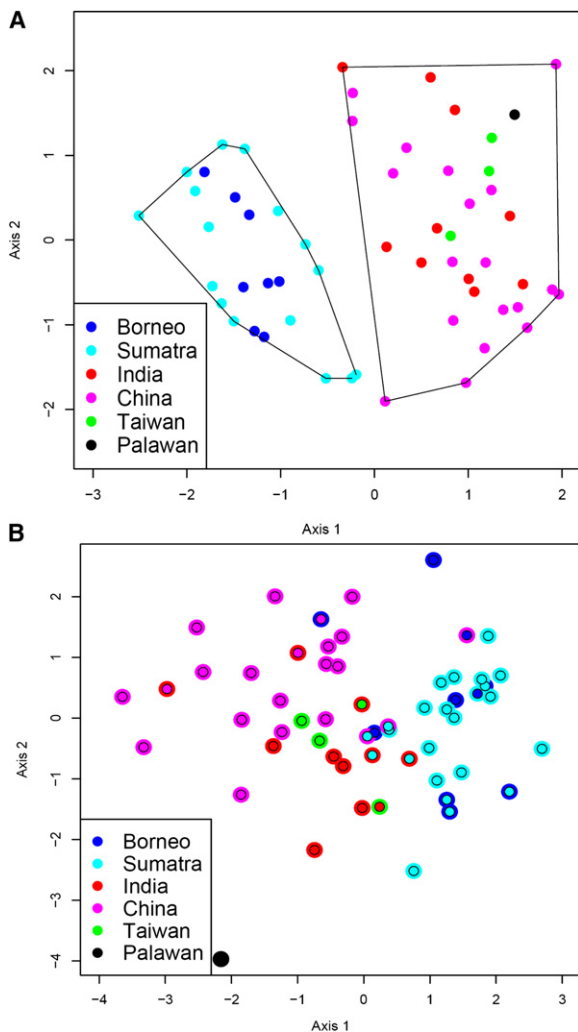
between mainland and Indonesian leopards. The discriminating ability of pelage characters other than cloud size to predict the sampling location among the six regions is, however, generally weaker. The proportion of the between-group variance explained by the first two axes is 82%. There is good separation of individuals from Borneo and Sumatra (including the Batu Islands) from the others (Figure 2B). The level of classification is generally good within these two groups, with only one individual from the Borneo and Sumatra group being classified outside (95% correct), and 5 out of 35 from the other group classified as belonging to Borneo and Sumatra (86%). The overall level of correct classification within each of the six sample locations is 42/57 (74%). The separation between the mainland and Indonesian forms is mainly on the first discriminant, and the characters that make the most contribution to being classified as mainland are a tawny pelage, thin neck stripes, and lighter pelage (based on coefficients of  $-1.92$ ,  $1.07$ , and  $-0.86$ , respectively; Table S3).

In summary, we have found in a multivariate analysis that two clusters of clouded leopards can be identified on the basis of their pelage characters. One group comes from mainland Asian populations (with large cloud size), and the other comes from Indonesia (small clouds). The pelage characters other than cloud size do not give rise to two clusters but, in a discriminant analysis, can be used for distinguishing between mainland and Indonesian forms quite accurately.

A potential caveat that needs to be borne in mind is the occasional presence of trade skins, which may affect the conclusions here, and also any future molecular analyses based on these museum specimens. Skins (often without any associated skeletal material) would have been collected by local people for trading either locally or farther afield, which European collectors would have obtained. An example of a trade skin is the specimen from Palawan in the Philippines (BMNH.1898.3.11.3), where this species has not been recorded. Its large cloud pattern would suggest that it has been traded from the mainland, probably China.

How do the results here impinge on the earlier hypothesis of four subspecies? There was no support for the *macrosceloides* subspecies from India and Nepal, based on ten samples (Table 3). All three specimens from Taiwan are also of the mainland form. The original diagnosis was made on the basis that the *brachyura* subspecies had a shorter tail, although subsequent studies suggest that this is not a reliable characteristic [12, 13]. Our examination suggests that the holotype is a trade specimen with an incomplete tail. It is worth noting that Taiwan was connected to the mainland during the last Ice Age, which reached its peak circa 20,000 years ago.

It has been suggested that animals from the Malay Peninsula are also of the *diardi* type (i.e., small clouds) [1, 12]. The Malay Peninsula fauna often shows a close affinity to that of Sumatra, owing to land connections caused by lower sea levels during glaciations. The



**Figure 2. Discriminating between Clouded Leopards**  
**(A)** Nonmetric multidimensional scaling of ten pelage characters from 57 clouded leopards, and scaling shows complete separation of mainland and Indonesian populations as judged by convex hulls drawn around the two groups of points.  
**(B)** A plot of the first two discriminant variables based on nine pelage characters (excluding cloud size, CSI) for 57 clouded leopards. The outer color of each point refers to the actual location of the individuals. The color of the inner circle refers to the predicted location from the discriminant analysis.

narrow Isthmus of Kra is a well-known biogeographical barrier that has isolated many forms from the rest of southeast Asia [14]. Therefore, it might be reasonable to assume that clouded leopards in Malaya are the Indonesian form. Our only specimen from Negri Sembilan, Malaya (BMNH.1908.7.20.76) is of the mainland form. Further specimens need to be examined from the Malay Peninsula south of the Isthmus of Kra before our hypothesis concerning the distribution of the mainland form there can be confirmed.

Groves [8], in the context of primate taxonomy, has strongly advocated the use of the PSC for taxonomic studies of this kind. The PSC relies on recognition of species when populations are 100% diagnostically distinct with at least one characteristic and has been

applied in a wide range of taxa (reviewed in [6]). On the basis of the cloud sizes on clouded-leopard pelages, all mainland (including the Taiwanese *brachyura*) clouded leopards are fully distinguishable from Indonesian animals, and hence, they should be recognized as distinct phylogenetic species (Figure 3). Therefore, we recommend the recognition of two species of clouded leopard as diagnosed below:

*Neofelis nebulosa* (Griffith, 1821 [15]), including *Felis macrosceloides* (Hodgson, 1853 [16]), *Leopardus brachyurus*, (Swinhoe, 1863 [17]).

Distribution: Mainland Asia, including the Nepal, Sikkim, Assam, Burma, China, Indochina and Malaya, and the island of Taiwan.

Diagnosis: On shoulders, large cloud-like markings, which extend the full depth of the flank and slope back from the dorsal midline, with mostly few spots within the clouds; a partially double dorsal stripe; pale, often tawny ground coloration (Figure 3).

*Neofelis diardi* (Cuvier, 1823 [18]).

Distribution: Sumatra and Borneo, including the Batu Islands.

Diagnosis: On shoulders, small irregular cloud-like markings, which form two or more rows that are arranged vertically from the dorsal midline on the flank, with frequent spots within clouds; ground coloration that is overall grayish yellow or gray hue; a double dorsal stripe (Figure 3).

The recent molecular analysis by Buckley-Beason, et al. ([9], this issue of *Current Biology*) strongly supports our hypothesis, although their study lacked samples from Sumatra. This revision has important implications for the conservation of the clouded leopard. Firstly, rationalization of the mainland subspecies allows for a more flexible approach to management of both wild and captive populations. Currently, all captive animals in western zoos and collections are of the mainland form, even if their geographical origin is unknown (most probably originate from China and southeast Asia). However, the occasional animal of North Borneo origin has been imported into zoos on mainland Malaysia and Singapore in recent years. Similarly, clouded leopards of Vietnamese origin have been imported into a zoo on Java in the past, but there is no evidence that any of these animals have ever contributed to the current captive gene pool. In the case of the importations into mainland Malaysia specifically, it may be indicative of the species being more severely threatened than its current IUCN Red Data List category (Vulnerable) suggests, at least locally, because the zoos concerned are within the putative current range of the clouded leopard. On Taiwan, the species has not been reported for some years, and at best it is thought to be very close to extinction [19, 20]. If the Taiwanese wildlife authorities were to consider reintroducing the species to the island, our results indicate that using animals of mainland Chinese origin would be taxonomically appropriate even if the species still occurred in isolated pockets of habitat on Taiwan. Our finding that the Indonesian clouded leopard represents a distinct species raises its conservation importance, particularly given the current high rate of habitat destruction in Sumatra and Borneo and the lack of



Figure 3. Morphological Variation between Mainland and Indonesian Clouded Leopards

Photographs of live clouded leopards from (A) Sumatra (Jambi Province: Zoological Society of London/LIPI), (B) Borneo (Sarawak, Lambir Hill National Park: Mohd. Azlan J.), (C) Sumatra (Jambi Province: ZSL/LIPI), (D) Borneo (Danum valley, Sabah; Siew Te Wong), (E) Cambodia (Seima Biodiversity Conservation Area, Mondulkin: Joe Walston, Wildlife Conservation Society Cambodia Program), (F) Thailand (Khao Yai National

any captive breeding program. We recommend urgently further molecular study for testing the hypothesis presented here.

#### Experimental Procedures

##### Material and Characters Scored

Skins from known localities were examined in museum collections (Table 1 and the Supplemental Data). Ideally, skull measurements would have been desirable. However, few skulls were available, and these were typically not associated with the skins, which were probably traded without skeletal material from local people. A number of pelage characters, derived partly from Pocock [12], were analyzed (Table 2). Seven of the characters were scored on a discrete ordinal scale. Coloration could not be scored on a single ordinal scale and was divided into three characters: yellow, tawny, or gray, which could either be “absent” or “present.” It was possible for a skin that was judged to have a combination of these colorations (e.g., yellow/tawny). Overall, a total of ten characters were scored for 57 individuals. Scores for the characters of all specimens used in this study are in Table S4. Because the scoring of coloration, brightness, and lightness was performed by visual assessment there is scope for these to be subject to recorder error. Use of published color tables was avoided because these are known to differ significantly between printings and give false impressions of the accuracy of colors. The use of photographs allowed side-by-side comparison of skins from different museums. Variation in lighting conditions may have affected these characters, but no systematic bias is expected. It is important to note that similar methods for scoring pelages in felids have previously been shown to be highly discriminatory and correlated to gene-frequency differences in molecular markers [21].

A total of 26 cells out of 570 (4.6%) were missing, owing to damage to skins. In order to carry out a multivariate analysis, the empty cells were filled with a  $k$ th nearest neighbor algorithm implemented by the `knn()` function in the EMV package under R [22]. For each individual with a missing value for a particular characteristic, the method works by finding  $k$  individuals that have been scored for that characteristic and that have the smallest Euclidean distance from the target individual as measured from the other characters. The missing character value is then replaced by the weighted average of the values in the set of  $k$  individuals, where the weights are inversely proportional to the Euclidean distance. In the analysis here, we chose  $k = 2$ . In this way, we use 95% of the data at the cost of some approximation, whereas if we excluded the skins that had missing data, a much larger proportion would be lost.

The correlation between pairs of coat-color variables were measured with the nonparametric statistic, Kendall's  $\tau$ , which varies from  $-1$  to  $+1$  depending on whether the association is negative, absent, or positive.

Ordinations on the ten characters listed in Table 3 were performed with multidimensional scaling implemented in isoMDS from the MASS library [23] in the R statistical package. Euclidean distances were used. This method tries to find a configuration in two dimensions such that the ranks of the distances between objects in this space match those of the objects. Ordinations were carried out with all ten pelage characters and also with these characters excluding cloud size (CSI). The convex hulls for two groups of points were drawn with the standard `chull()` function in R.

In addition, linear discriminant analysis was performed with the nine pelage characters, excluding cloud size. The difference between this method of ordination and the MDS is that the latter attempts to find a projection of points that match the distance between objects, whereas a discriminant analysis finds a projection of points that maximize the distance between the centroids of previously defined groupings—in this case, the six geographic regions given in Table 1. The analysis was performed with `lda()`

from the MASS library [23] in the R statistical package. The results of the analysis are independent of the scales used for scoring the coat characters.

##### Supplemental Data

Supplemental Data include additional discussion, one figure, and four tables and can be found with this article online at <http://www.current-biology.com/cgi/content/full/16/23/2377/DC1/>.

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