

Combining data from multiple sources: A cautionary tale

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INTRODUCTION

With the increasing numbers of instruments available for collecting linear and three-dimensional (3D) data, and with more published and on-line morphometric datasets, many studies now include data pooled from multiple observers and methods. Although several researchers have examined inter-observer and inter-method error (e.g., Badawi-Fayad and Cabanis, 2007; Tocheri et al., 2011; Shearer et al., 2014), this work has focused on a limited number of methods and/or specimens. Additionally, these analyses have not explicitly compared whether different observers, using a variety of methods, obtain similar results studying the morphology of the same specimens, including recovering the same relationships among individuals.

Here we compare data collected on the same specimens by two observers using four methods to determine the extent to which intra- and inter-observer as well as inter-method error influence the outcome of statistical analysis.

MATERIALS AND METHODS

Sample - Two observers (CAR and CET) collected data at least twice (i.e., 'trials') on 14 cranial specimens from 12 species ranging in size from *Callicebus* to *Gorilla*.

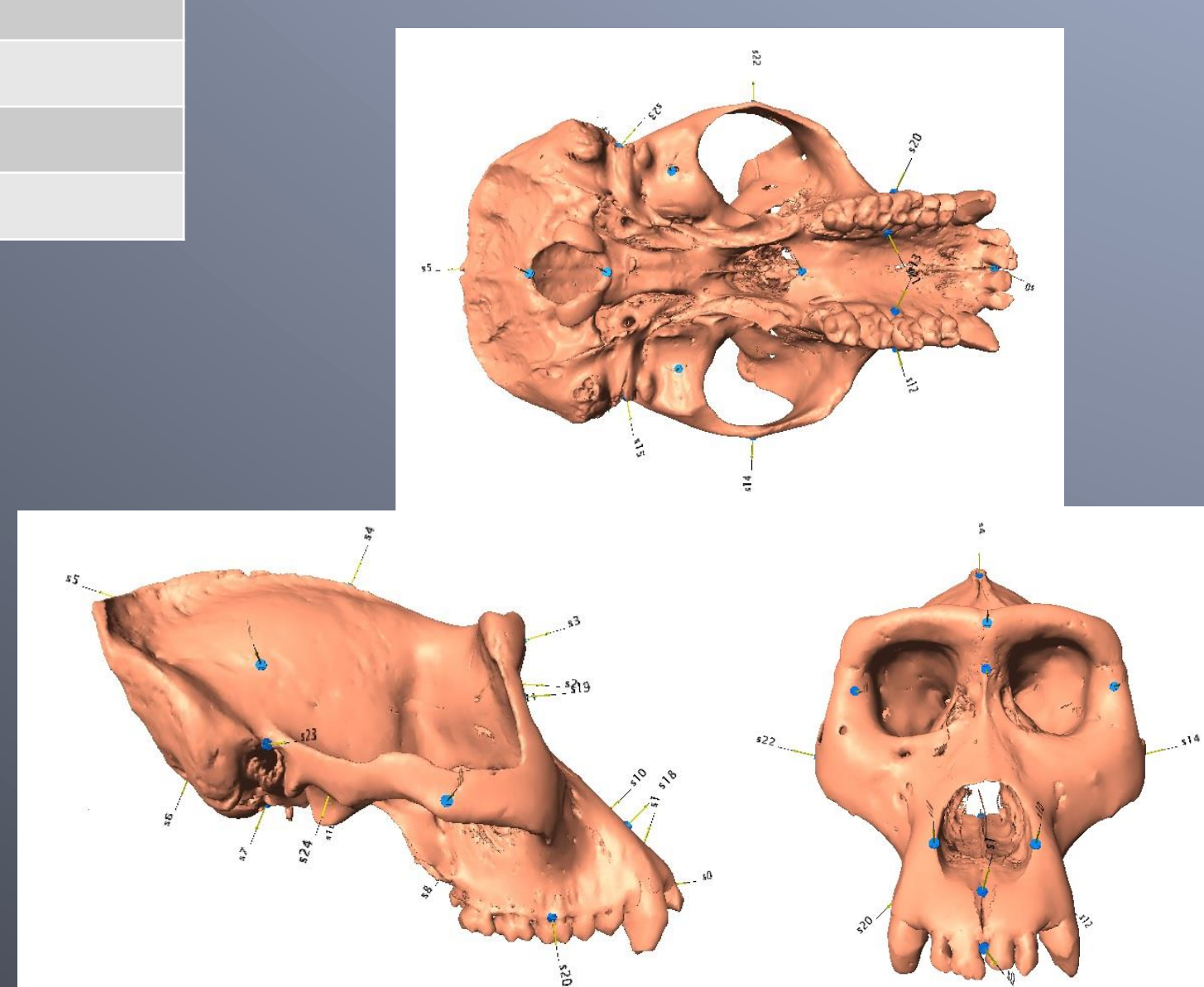
Data collection - Four data collection methods were employed: 1) 15 linear measurements were collected using Mitutoyo digital calipers; and 26 3D landmarks were collected 2) directly on the specimen using a Microscribe-3DX (MS) digitizer, on 3) scans collected using a NextEngine (NE) laser scanner, and on 4) surface models created from microCT scans generated using a GE Phoenix v|tome|x s 240 high resolution scanner (CT) in Landmark 3.0.0.6 (Wiley et al., 2005). 3D data were both converted to linear distances and used in 3D analyses.

2D data analysis - A nested ANOVA was run on the linear data to explore the extent of variance explained by taxon, specimens, observer, method, and trial.

3D data analysis - 3D data were analyzed using geometric morphometric methods. In addition to an analysis of all trials of all specimens, six analyses (broken down by observer and method) were run on the 11 specimens for which CT scans were available. For each analysis, specimens were superimposed and a PCA was performed in Morphologika (O'Higgins and Jones, 1998) to examine whether all trials of the same individual grouped together and whether the distributions of specimens in the six PCAs of the data collected by each observer and each method were similar. Procrustes distances were calculated among trials of the same specimen, among observers, among methods, and all combinations therein. We compared these distances to distances between specimens in the same species, the same genus, and among genera and superfamilies that were collected by the same observer using the same method. Procrustes distances were also used to generate UPGMAs depicting these similarities in morphospace.

Sample (* - no CT scan available)	
Taxon	Specimen number(s)
<i>Aotus azarae</i>	AMNH 36508
<i>Callicebus cupreus</i>	AMNH 72141, 72143, 75987, 75988
<i>Allenopithecus nigroviridis</i>	AMNH 86856
<i>Cercopithecus albogularis</i>	AMNH 27717*
<i>Cercopithecus mitis</i>	AMNH 52355*
<i>Macaca hecki</i>	AMNH 152890
<i>Macaca sylvanus</i>	AMNH 202391
<i>Papio hamadryas anubis</i>	AMNH 82185
<i>Gorilla gorilla</i>	AMNH 99-9686
<i>Nomascus leucogenys</i>	AMNH 87251
<i>Pan troglodytes</i>	AMNH 167344*

Linear measurements	
Maximum cranial length, height, and breadth	Maxillary breadth
Nasal height and breadth	Palate breadth and length
Biorbital breadth	Biarticular breadth
Bizygomatic breadth	Biporionic breadth
Mandible length (on cranium)	Foramen magnum length
Facial length	

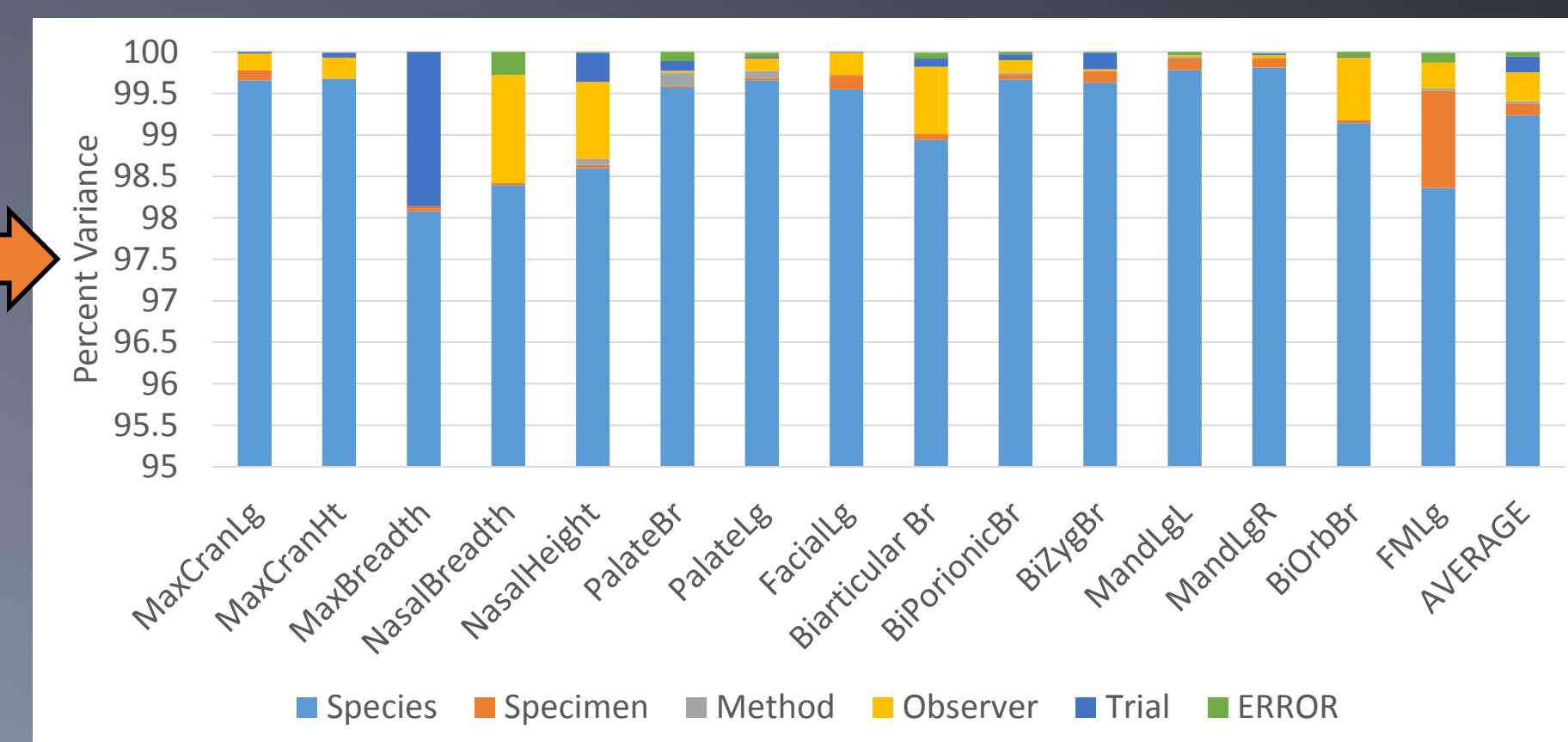


Landmarks	
Prosthion	Orale
Nasospinale	L/R alare
Nasion	L/R ectoconchion
Glabella	L/R ectomolare
Bregma	L/R endomolare
Opisthocranium	L/R zygion
Opisthion	L/R porion
Basion	L/R AE midpoint
Staphylion	L/R eurion



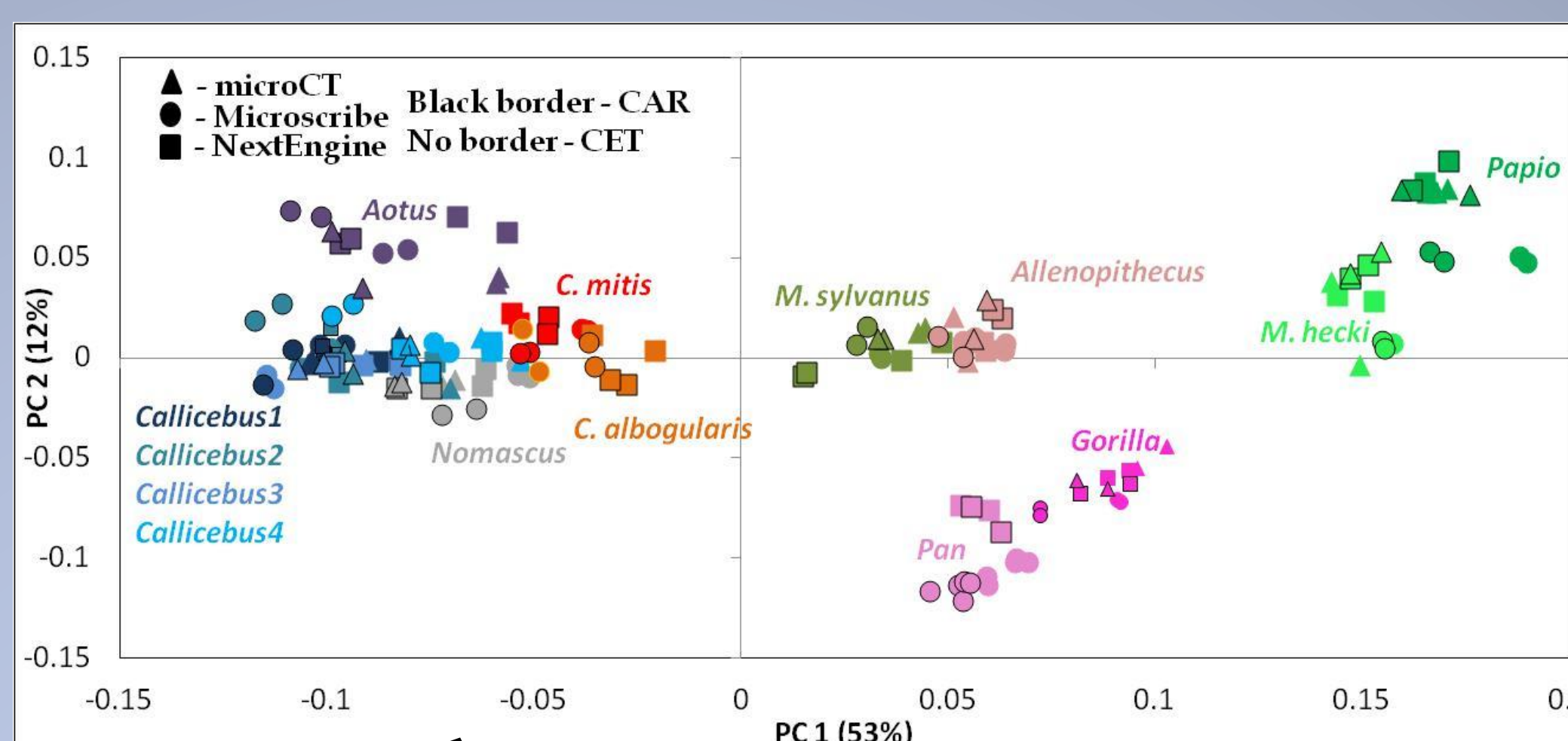
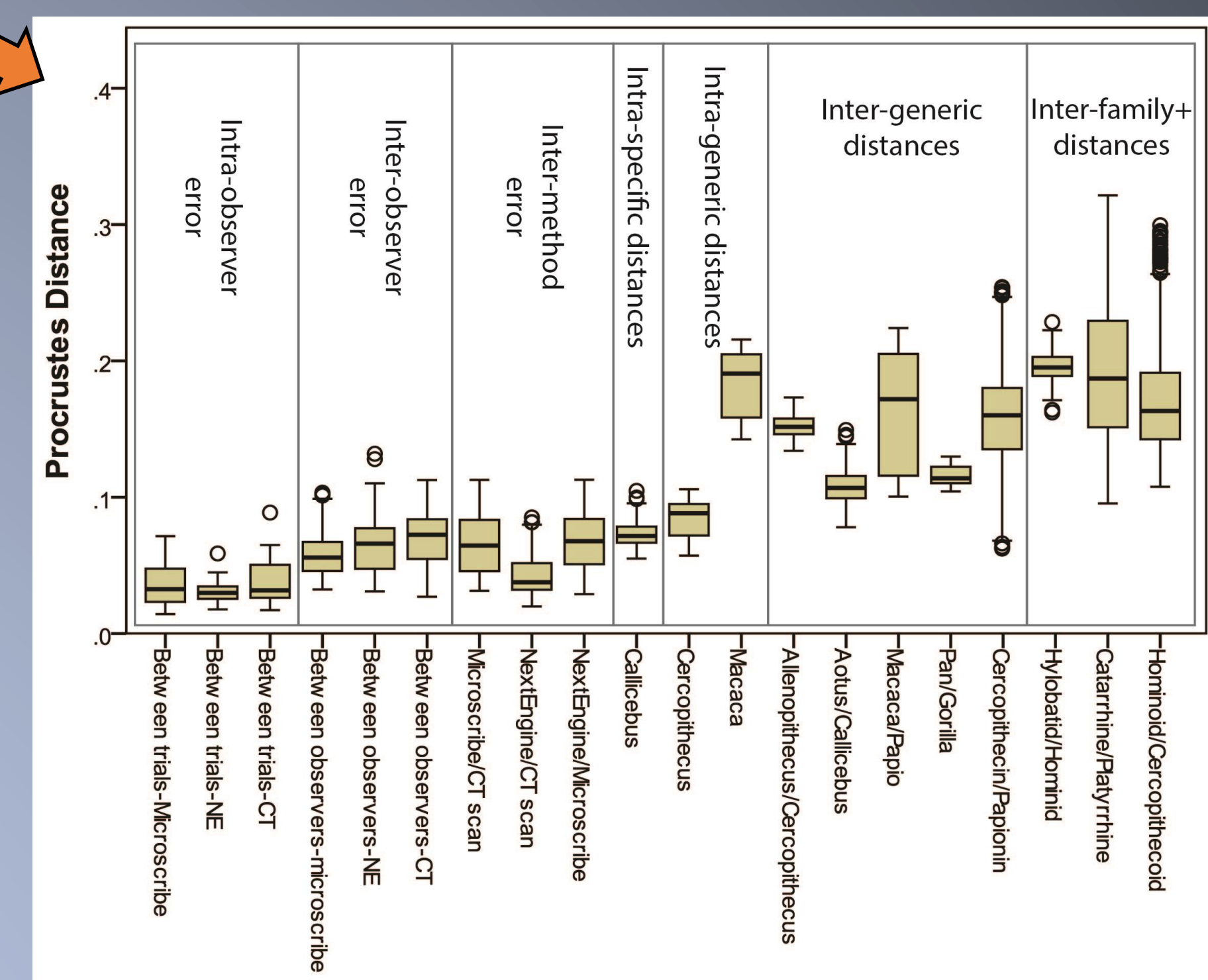
RESULTS- 2D DATA ANALYSIS

- By far the most variance in the linear data was at the level of species (98.08-99.81% of variance), although this is at least partly due to the large size differences among the specimens, with the second most variance at the level of observer and then trial. Error due to use of different methods was minimal.

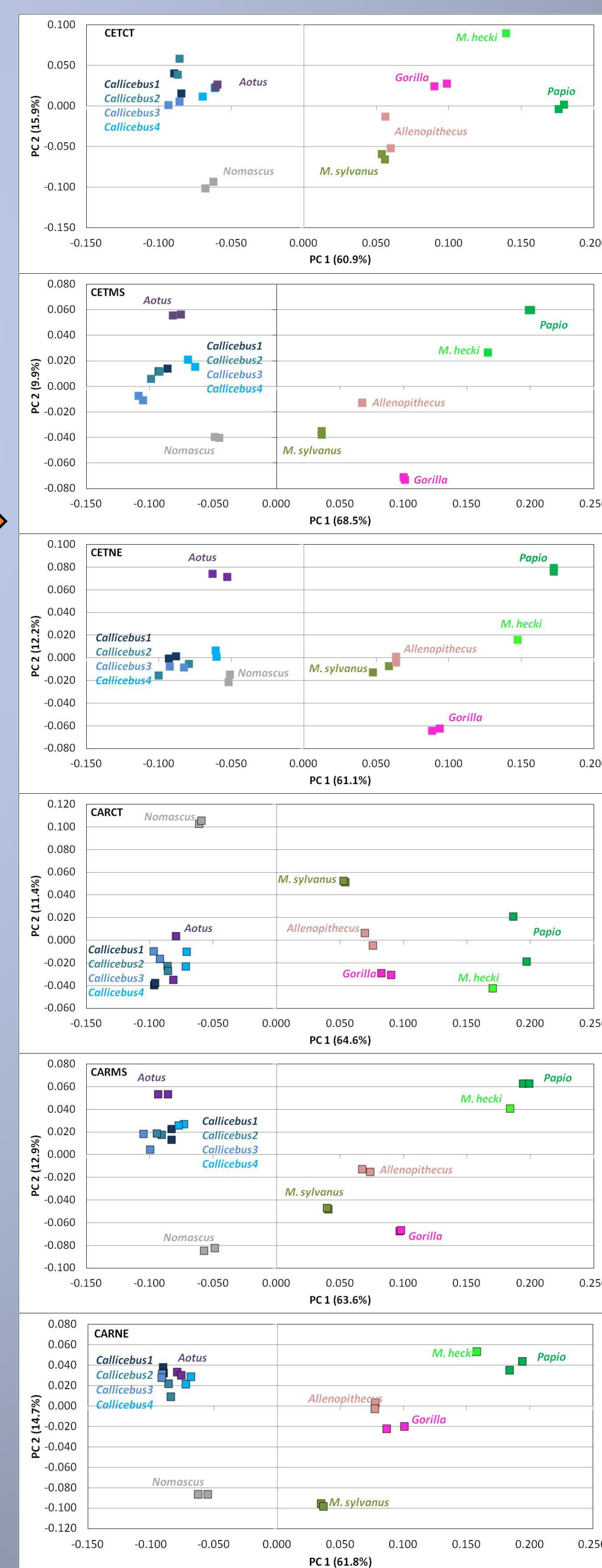


RESULTS- 3D DATA ANALYSIS

- Procrustes distances were smallest between trials of specimens measured by the same observer using the same method (intra-observer error). Inter-observer and inter-method error are similar to intra-specific distances and only slightly less than intra-generic distances for *Cercopithecus*, with inter-observer error higher than inter-method error in general (although Microscribe to other method distances are higher, likely due to a time delay in data collection).

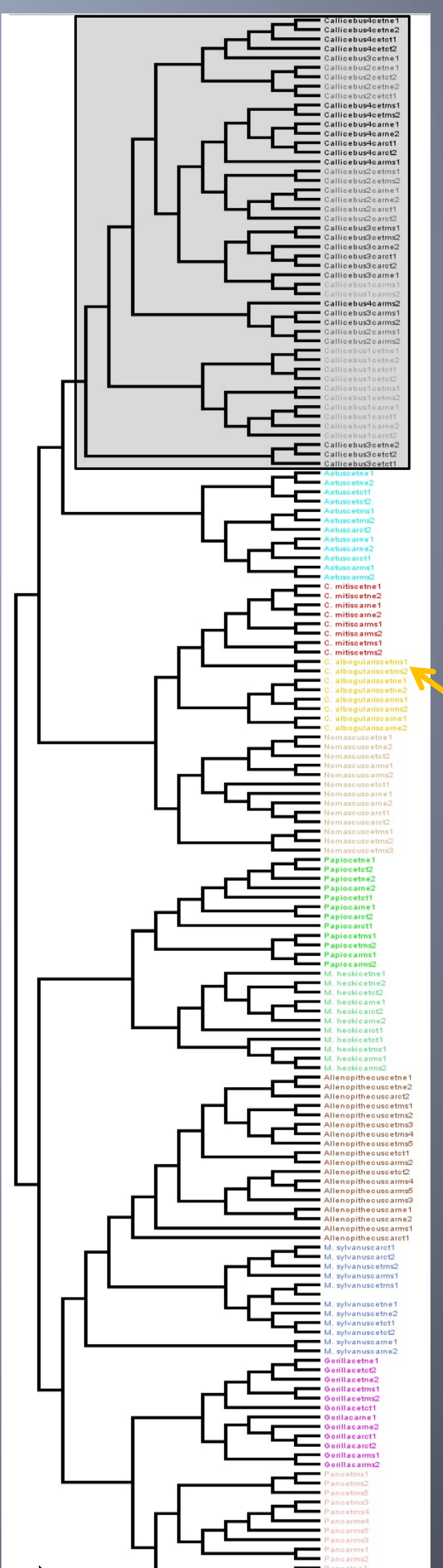


- In the PCA with all data included, taxa were generally well separated and different trials of the same specimen typically grouped together on the plot of PCs 1 and 2. Notably, the four *Callicebus* specimens overlap substantially such that these individuals are not consistently separated from one another. There is also some overlap between the trials of the two *Cercopithecus* taxa.



- Specimen distributions are similar in the six PCAs representing observer and method. On PC 2, the positions of the platyrrhine specimens and *Nomascus* are fairly consistent, with more variation in the positions of catarrhine species on the positive end of PC 1. However, the three PCAs derived from data collected by each observer using different methods are, in general, more similar to one another than to those derived from data collected by the other observer using the same method.

- Trials of each specimen generally grouped together in the UPGMA tree. The most notable exception to this is with the *Callicebus* specimens (in the lightly shaded box) for which there were no consistent groupings. Similarly, two trials of *Cercopithecus albogularis* group with the *Cercopithecus mitis* trials (see yellow arrow). Similar results were obtained with UPGMA trees were generated for different observers using different methods, though in the plot of CAR's Next Engine data *Macaca sylvanus* groups with *Nomascus* as the sister to the platyrrhines rather than with the other catarrhines (plot not shown).



CONCLUSIONS

Our results suggest **researchers should be cautious when compiling data from multiple methods and/or observers**, especially if their analysis focuses on intraspecific variation or closely related species, since these patterns may be obscured by inter-observer and inter-method error. Conducting inter-observer and inter-method reliability assessments prior to the collection of data (and collecting all data within a relatively short amount of time) is recommended, and compiling data from published sources should probably be avoided for studies of closely related individuals. This problem may be somewhat alleviated in the future with the greater availability of 3D scans in online repositories (e.g., MorphoSource.org).

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