

Supplementary Information for

Importance of using genetic data to inform conservation captive breeding: A tale of two species of threatened macaws (*Ara* spp.)

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Figures S1–11

Data S1 caption

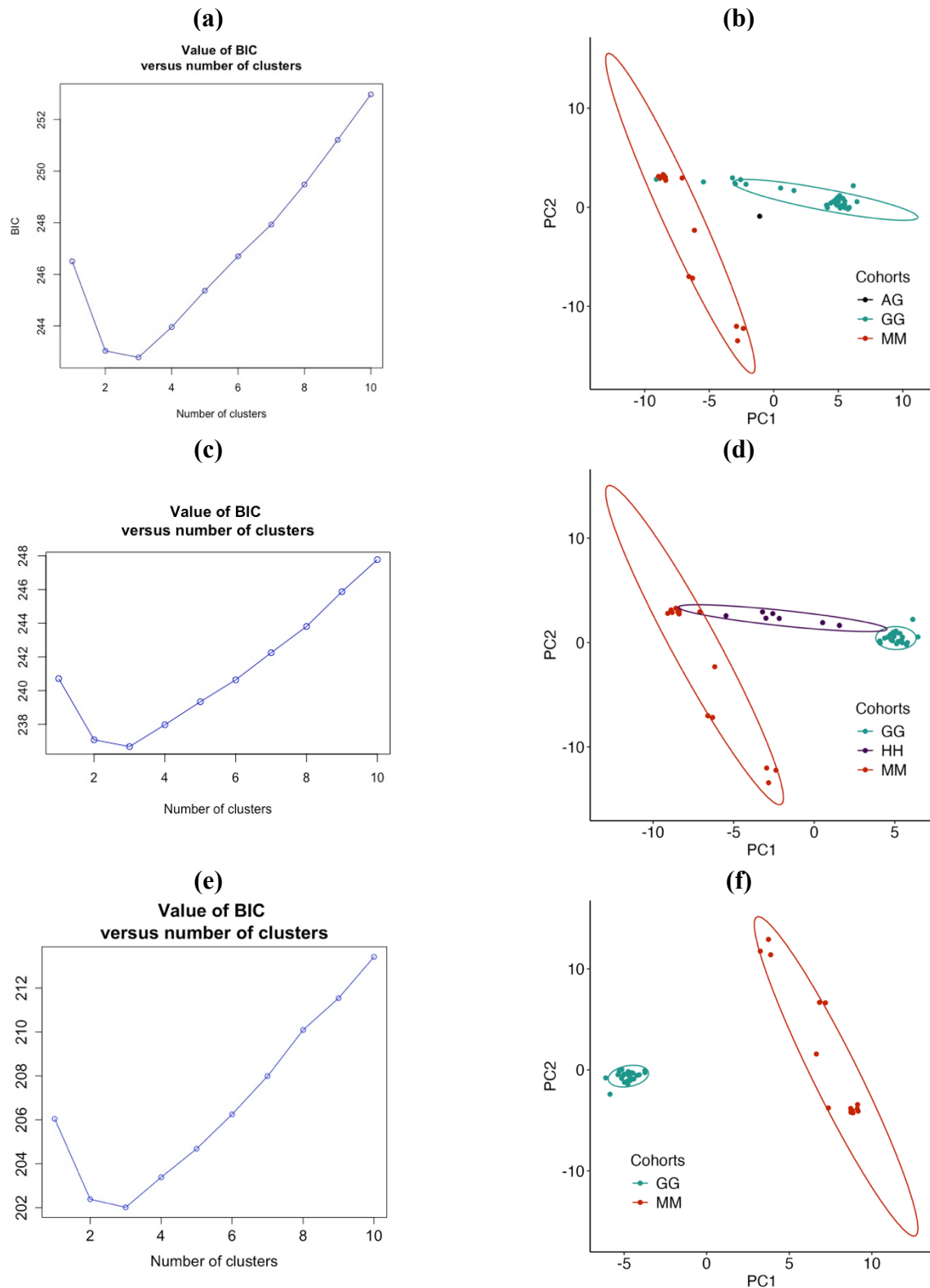


Figure S1. Nuclear SNP cluster analyses (a), (c), (e) and principal component plots (b), (d), (f) among parrot samples. Samples are *a priori* grouped as (b) Great Green Macaw (*Ara ambiguus*; GG, $n = 31$), Military Macaw (*Ara militaris*; MM, $n = 14$), and Grey Parrot (*Psittacus erithacus*; AG, $n = 1$) as outgroup; (d) Great Green Macaw (GG, $n = 23$), Military Macaw (MM, $n = 15$), and their potential hybrids (HH, $n = 7$); and (f) Great Green Macaw (GG, $n = 23$) and Military Macaw (MM, $n = 15$).

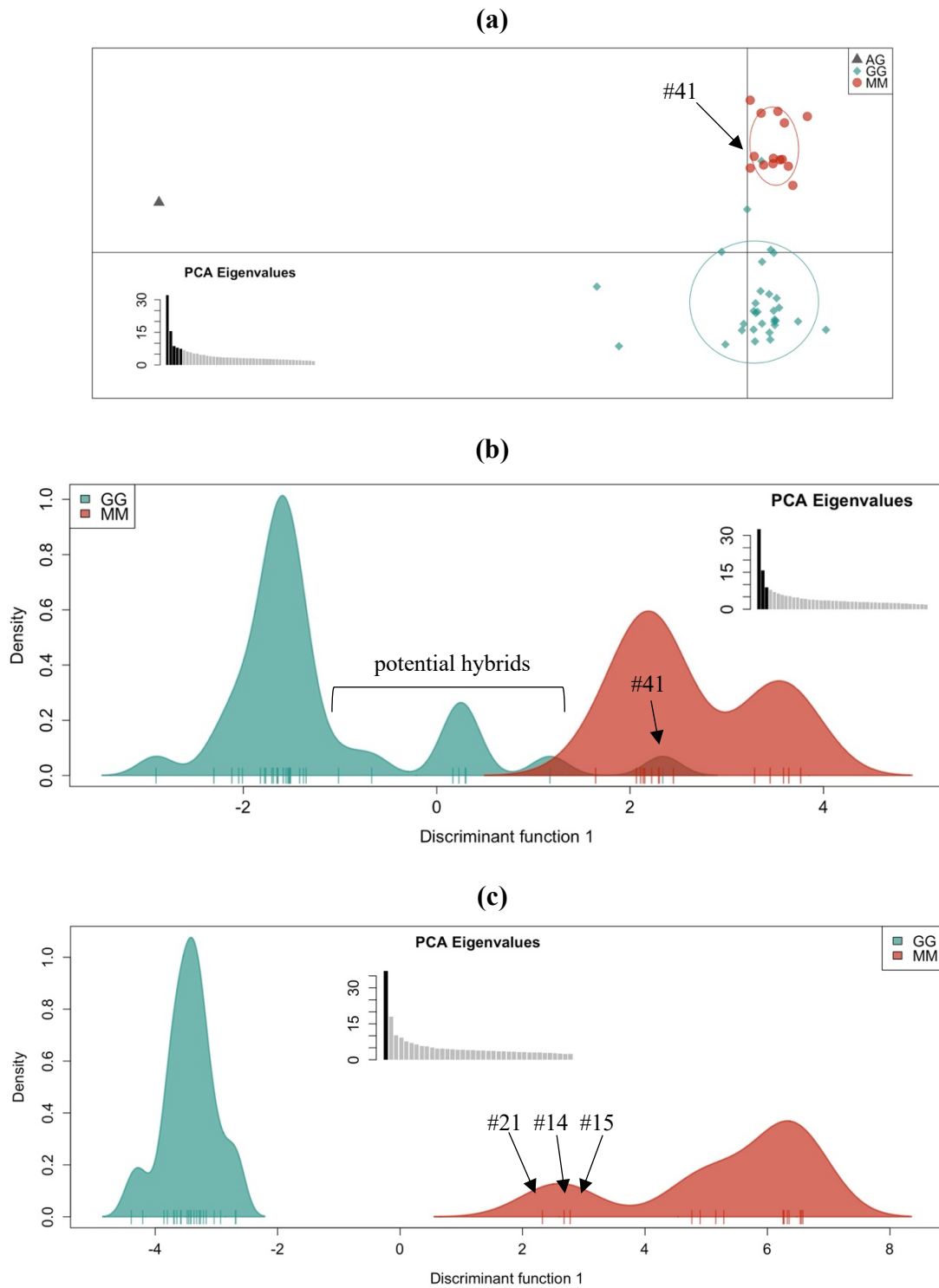


Figure S2. Grouping of parrot samples by discriminant analysis of principal components (DAPC) using 4143 nuclear SNPs. (a) Samples were *a priori* identified as Great Green Macaw (*Ara ambiguus*; GG, $n = 32$), Military Macaw (*Ara militaris*; MM, $n = 15$), and Grey Parrot (*Psittacus erithacus*; AG, $n = 1$) as outgroup. Note that sample #41 was misidentified initially as GG but correctly assigned to MM by DAPC. (b) DAPC without the Grey Parrot. Misidentified sample #41 and potential hybrids are indicated. (c) DAPC with sample #41 assignment corrected and without hybrids, resulting in 23 GG and 15 MM individuals (#21, 14, and 15 possibly belong to the Bolivian subspecies, see Figure 3).

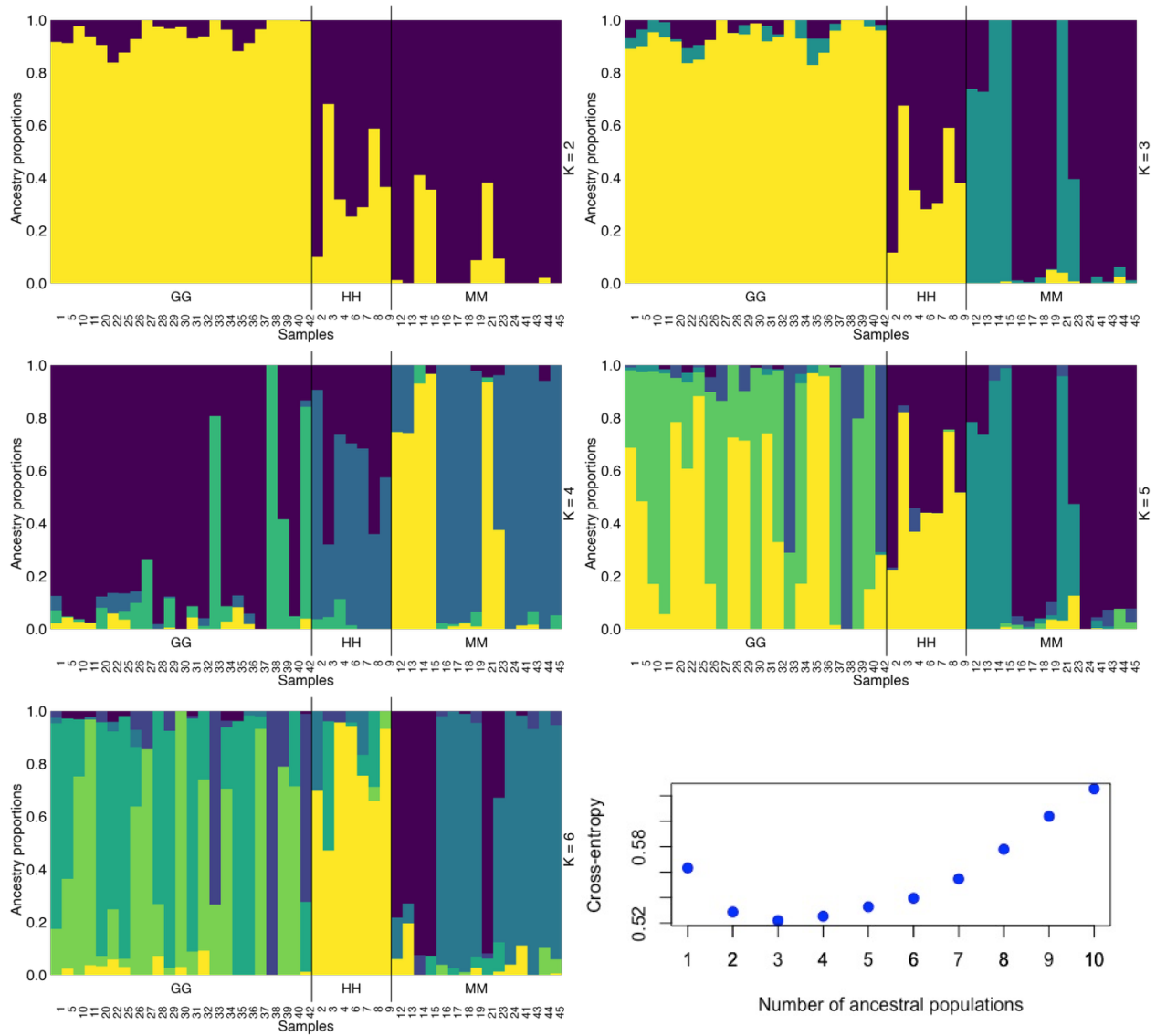


Figure S3. Admixture analyses with sparse non-negative matrix factorization (SNMF) among GG and MM samples (n = 45) using 4143 nuclear SNPs. Displayed are the individual ancestry coefficients for $K = 2$ – 6 clusters and the cross-entropy plot. Sample groupings represent Great Green Macaws (*Ara ambiguus*; GG, n = 23), Military Macaws (*Ara militaris*; MM, n = 15), and their hybrids (HH, n = 7).

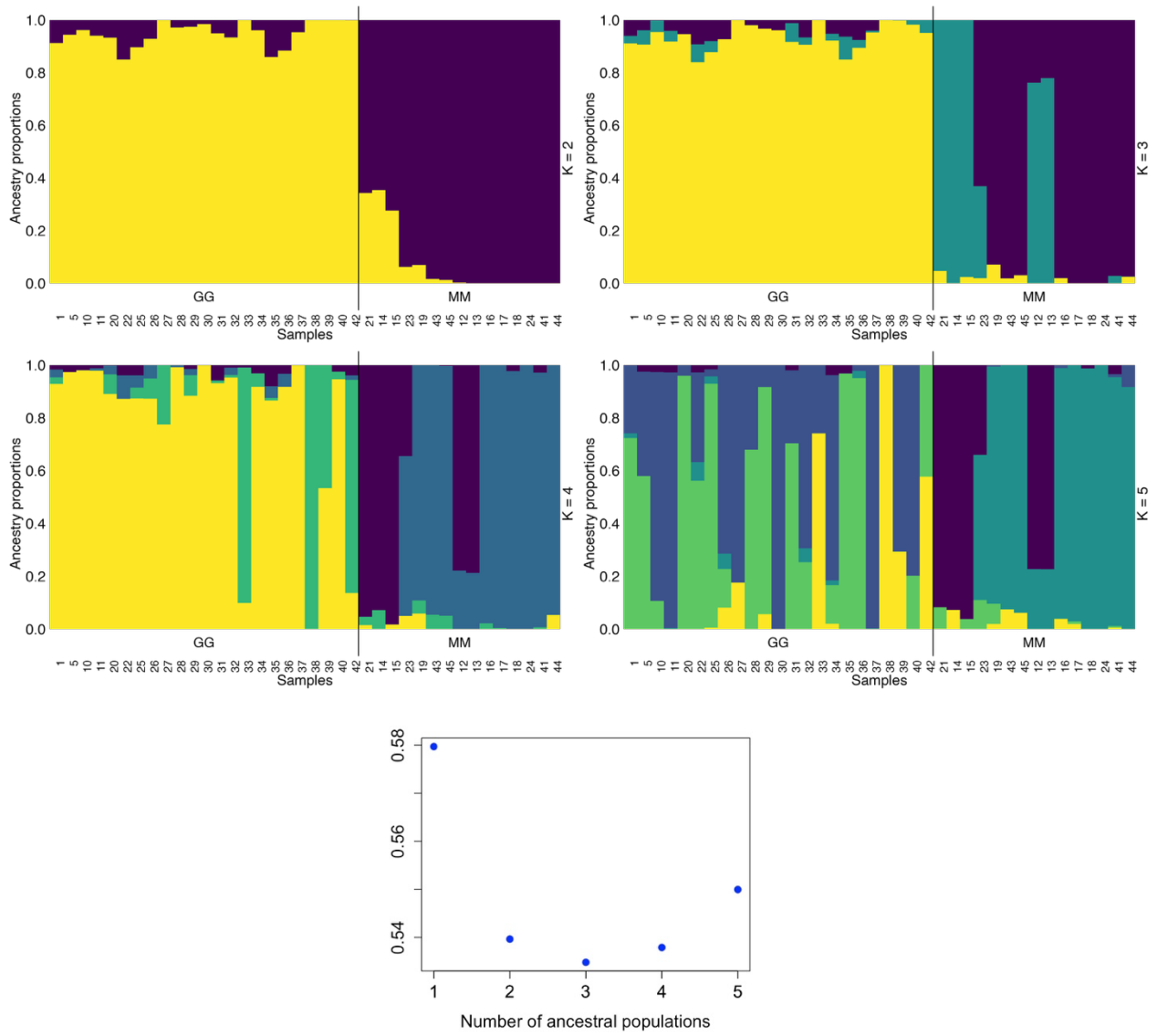


Figure S4. Admixture analyses with sparse non-negative matrix factorization (SNMF) among GG and MM samples ($n = 38$) after hybrids removed, using 4143 nuclear SNPs. Displayed are the individual ancestry coefficients for $K = 2-6$ clusters and the cross-entropy plot. Sample groupings represent Great Green Macaws (*Ara ambiguus*; GG, $n = 23$) and Military Macaws (*Ara militaris*; MM, $n = 15$).

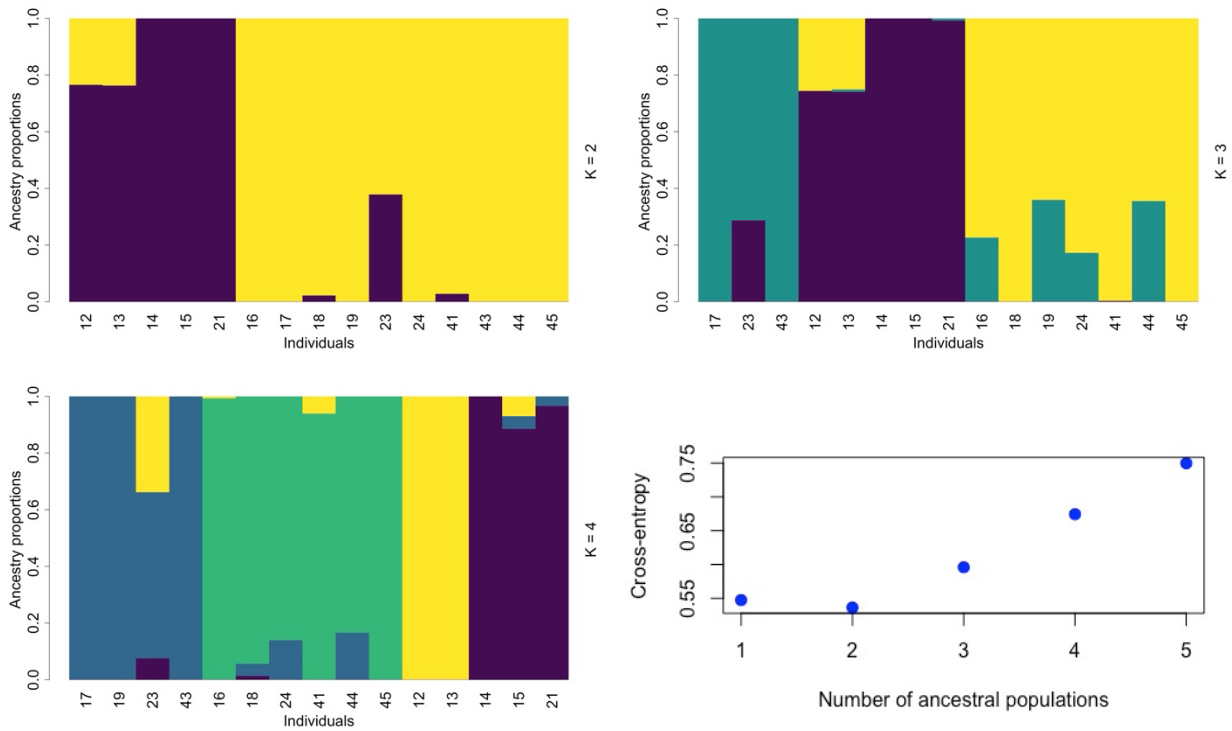


Figure S5. Admixture analyses with sparse non-negative matrix factorization (SNMF) among Military Macaws (*Ara militaris*; n = 15) using 4143 nuclear SNPs. Identified hybrids (see Figure S3) were excluded. Displayed are the individual ancestry coefficients for $K = 2-4$ clusters and the cross-entropy plot.

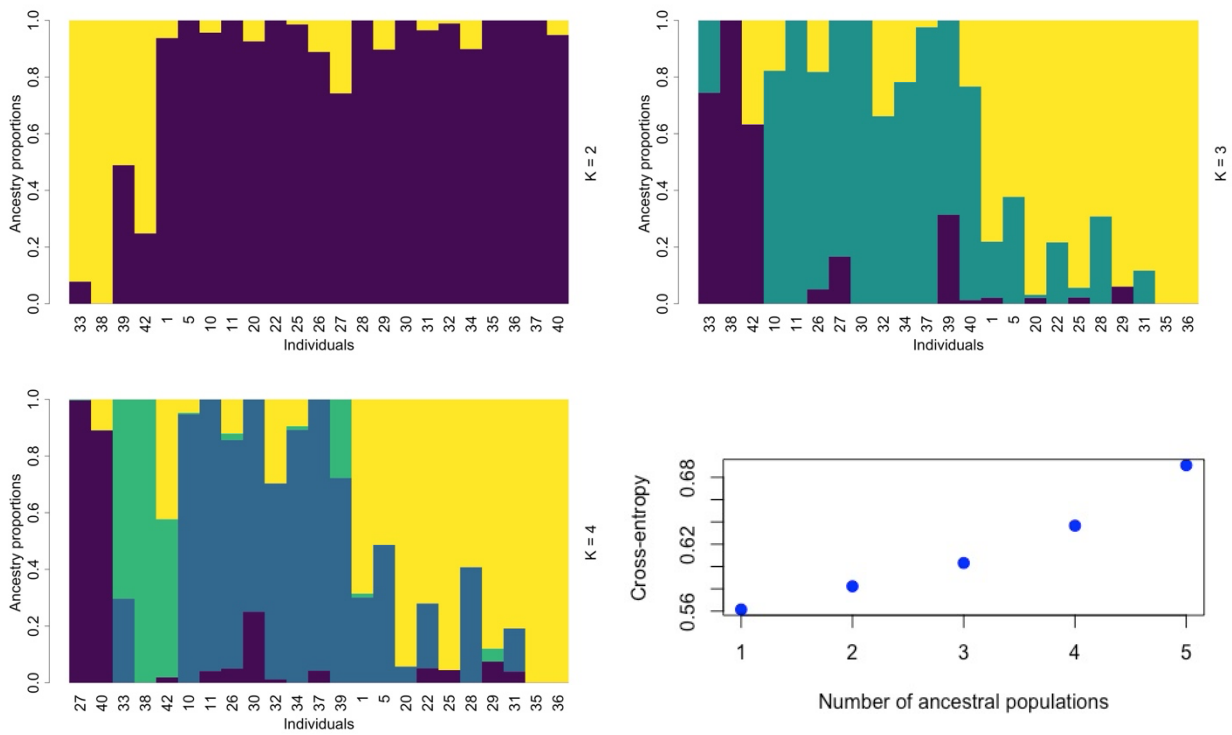


Figure S6. Admixture analyses with sparse non-negative matrix factorization (SNMF) among Great Green Macaws (*Ara ambiguus*; n = 23) using 4143 nuclear SNPs. Identified hybrids (see Figure S3) were excluded. Displayed are the individual ancestry coefficients for $K = 2-4$ clusters and the cross-entropy plot.

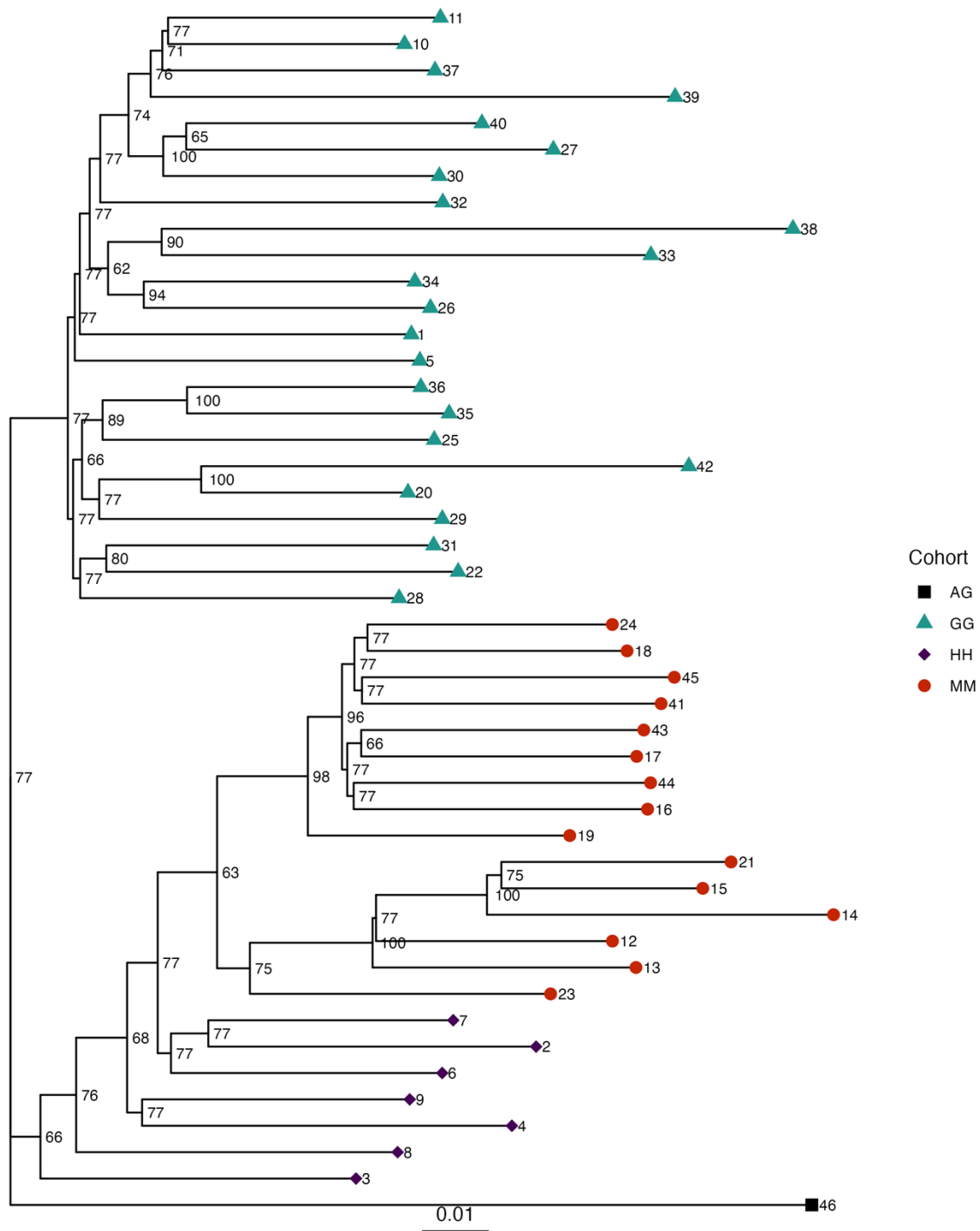


Figure S7. Neighbor joining tree using Nei's distance among Great Green Macaws (*Ara ambiguus*; GG, green triangles) and Military Macaws (*Ara militaris*; MM, red circles) and their hybrids (HH) using 4143 nuclear SNPs. Bootstrap values were calculated based on 1000 permutations, and the tree was rooted with a Grey Parrot sample (*Psittacus erithacus*; AG, black square). Potential hybrid individuals (HH, purple diamonds) were clustered intermediate between the two macaw species.

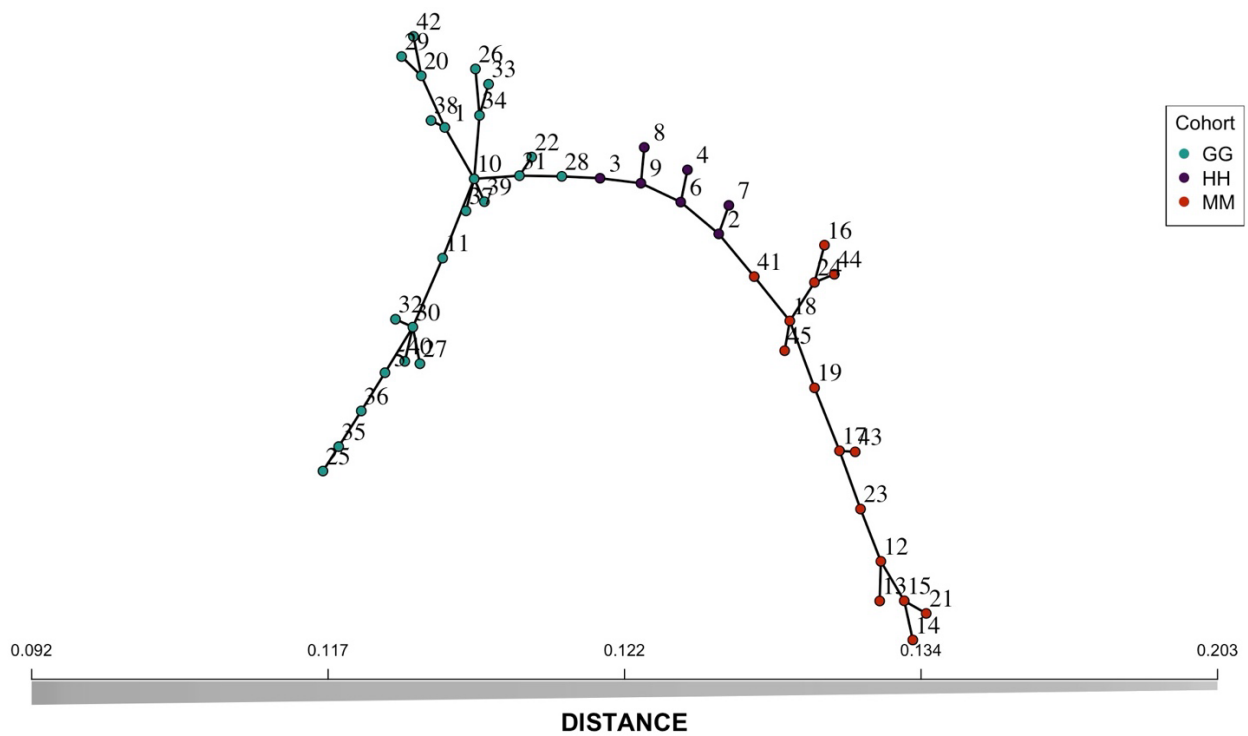


Figure S8. Minimum spanning network using Prevosti's genetic distance among Great Green Macaws (*Ara ambiguus*; GG), Military Macaws (*Ara militaris*; MM), and their hybrids (HH) using 4143 nuclear SNPs. The maximum Prevosti's genetic distance between all study individuals was 10%.

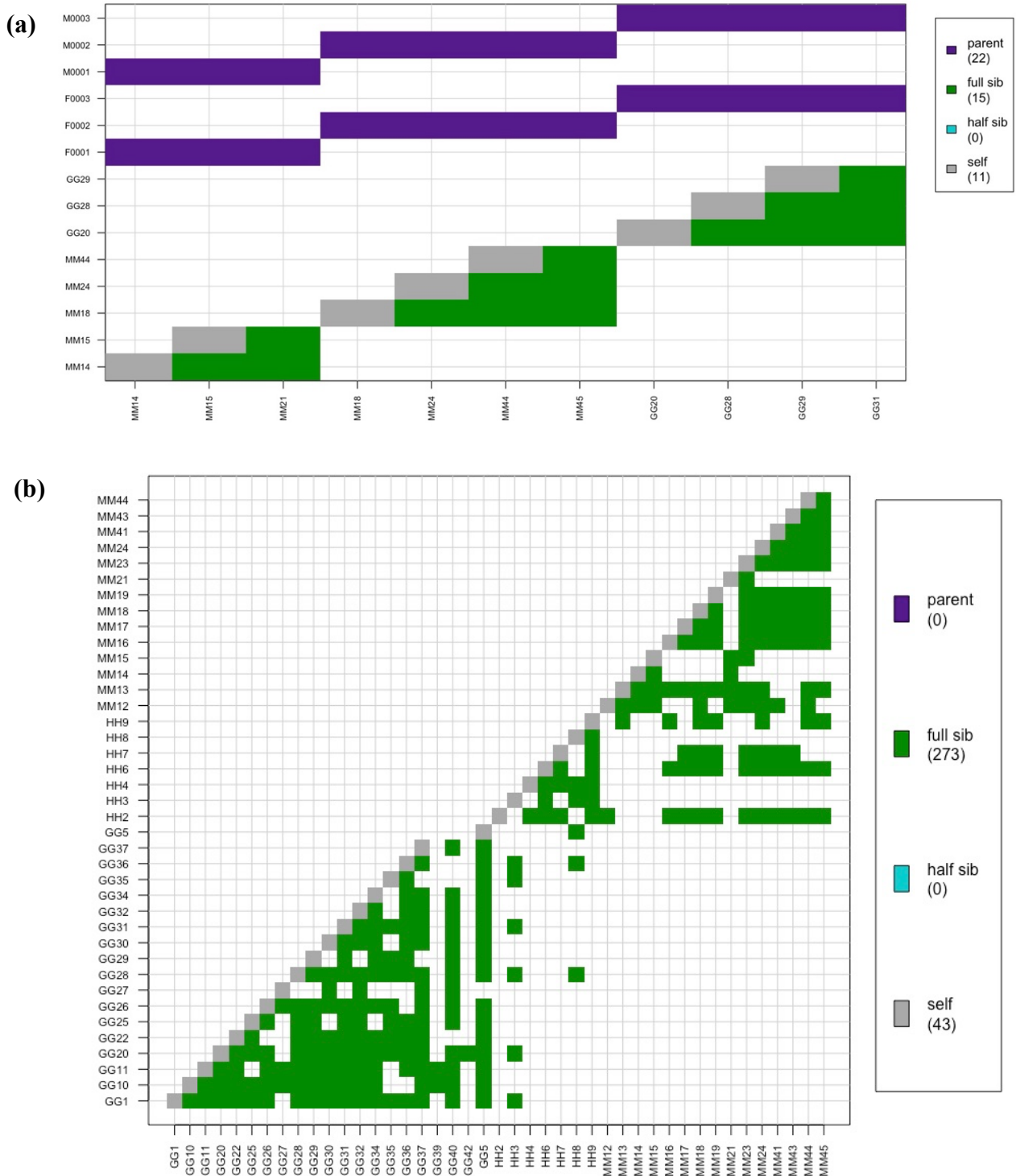


Figure S9. Pairwise kinship estimates among Great Green Macaws (*Ara ambiguus*; GG), Military Macaws (*Ara militaris*; MM), and their hybrids (HH) with Sequoia using 4143 nuclear SNPs. (a) Identified pairwise first order relationships (parent-offspring and full-siblings) between macaw samples labeled by unique IDs on the axes, only including individuals that passed the strict log-likelihood ratio thresholds for high-confidence pedigree assignment. The top seven individuals are “dummy” samples (where “M” indicates an inferred male parent and “F” an inferred female parent) generated during the analysis, representing the presumed parents of the sampled individuals. (b) Putative relatives identified using the “GetMaybeRel” function.

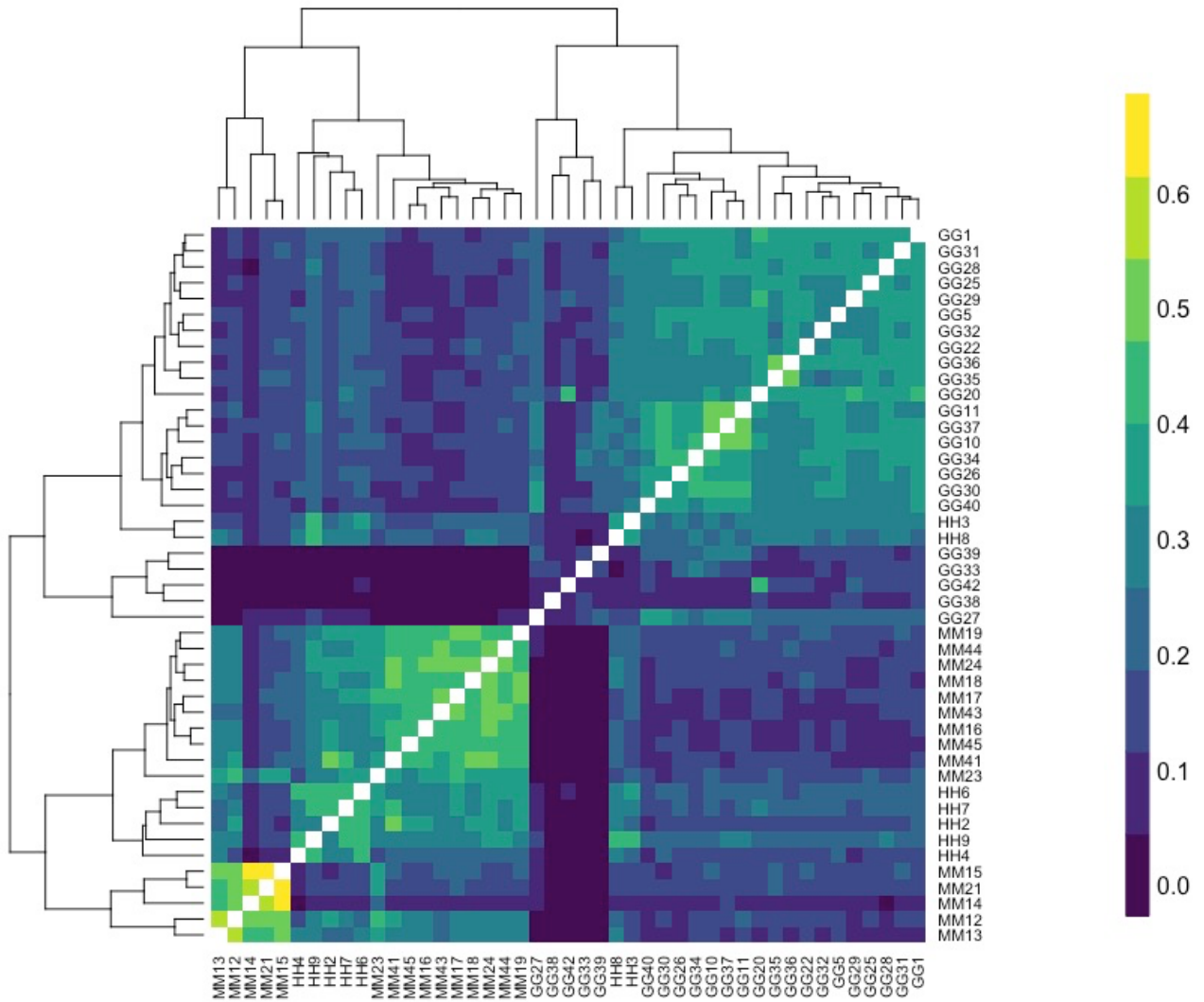


Figure S10. Pairwise kinship estimates (TrioML relatedness) matrix among Great Green Macaws (*Ara ambiguus*; GG), Military Macaws (*Ara militaris*; MM), and their hybrids (HH) plotted using hierarchical clustering of individuals.

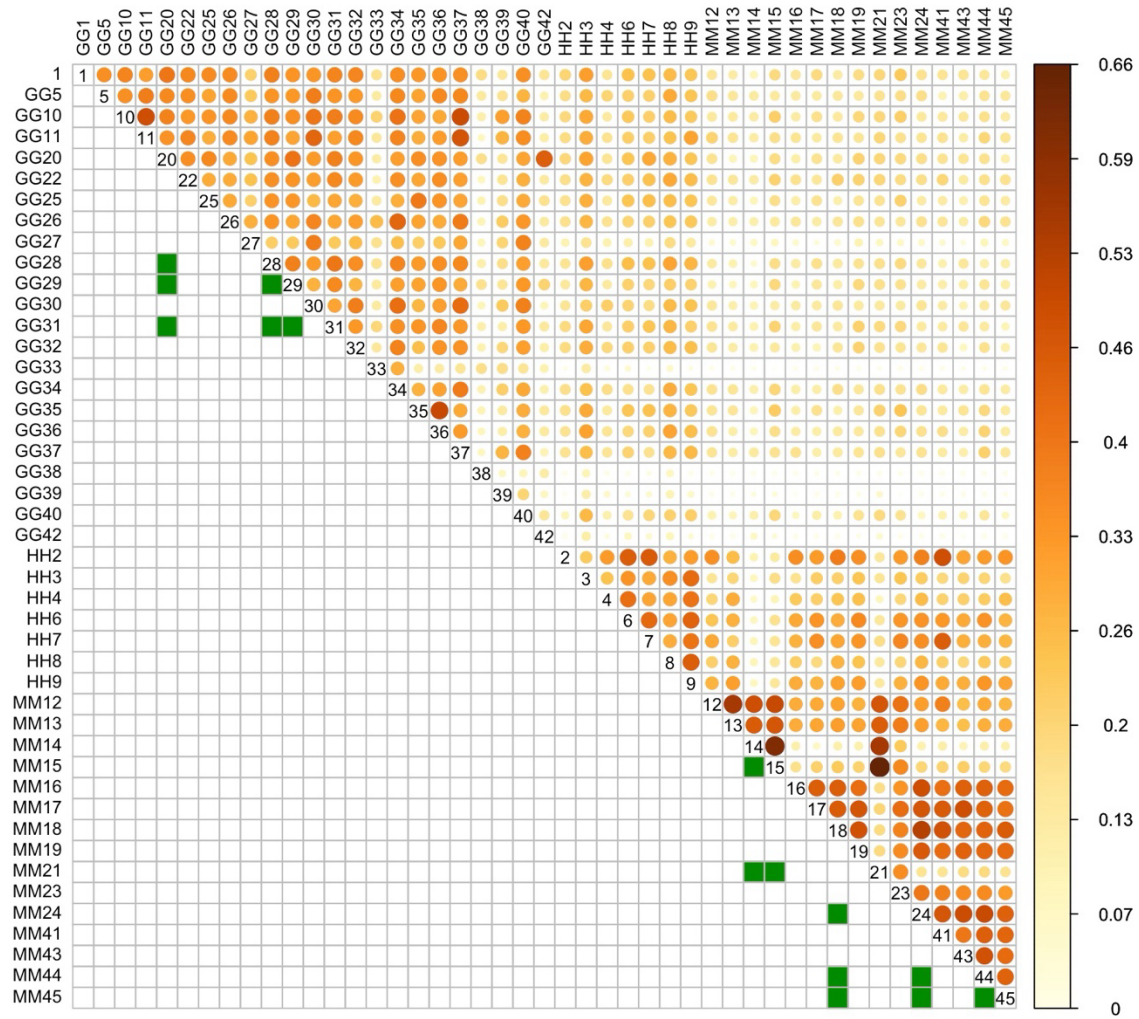


Figure S11. Pairwise kinship estimates among Great Green Macaws (*Ara ambiguus*; GG), Military Macaws (*Ara militaris*; MM), and their hybrids (HH). TrioML relatedness values are shown above the diagonal with values increasing from white (zero relatedness) to dark red (maximum relatedness) as shown to the right of the matrix, and first order kinship categories (full-siblings = green) assigned by Sequoia below the diagonal.

Data S1. Pairwise relatedness estimates of Wang, LynchLi, LynchRd, Ritland, QuellerGt, DyadML, and TrioML.