SUPPLEMENTARY INFORMATION

Specimens. The numbers of faecal and urine samples collected at different sites in the vicinity of Kisangani in the 2000 and 2003 expeditions are shown in Table 1. The sites correspond to the forest areas near villages depicted in Fig. 1b of the main text.

Table 1. Noninvasive urine and faecal sampling of wild chimpanzees near Kisangani, DRC

Site	Sample collection date	Urine samples	Faecal samples
Wanie-Rukula forest	1/00	10	34
Wanie-Rukula forest	2/03 to 4/03	9	53
Obiatuku forest	3/03 to 4/03	27	42
Parisi forest	5/03	0	2

Urine immunoblot analysis. Enhanced chemiluminescent Western immunoblots were performed as described, using scoring criteria previously established[1]. Reactivities to the HIV-1 envelope glycoprotein gp160 or to any three structural proteins were scored positive; the absence of viral bands was scored negative; and samples which did not meet either criterion were scored indeterminate. Unlike our usual protocol[1], urine samples from the 2000 expedition were collected in RNA*later* at a 1:10 to 1:20 dilution. RNA*later* is a high salt solution (25 mM Sodium Citrate, 10 mM EDTA, 70 g ammonium sulfate/100 ml solution, pH 5.2) that preserves nucleic acids[2], but generally precludes immunoblot analysis. However, we found that antibodies could be recovered from urine/RNA*later* mixtures by freezing the samples (–70°C), centrifuging at high speed (23,500×g for 1 hr at 4°C), and resuspending the pellet in Phosphate Buffered Saline (PBS), followed by immunoblot analysis as described[1].

SIVcpz viral RNA detection. RNA was extracted from faecal RNA*later* suspensions using the RNA*queous* Midi-Kit (Ambion; TX). Diagnostic reverse-transcriptase polymerase chain reaction (RT-PCR) was performed as described[1]. The *gp41*/nef primer set amplifies a 740- to 790-bp fragment at the *gp41*/nef overlap[3]. The *gag* primer set amplifies a 460-bp fragment in the p24 region and consists of first-round primers CPZ-GAG-F1 (5'-RGA AGT MAT CAA TGA GGA AGC-3'), CPZ-GAG-R1 (5'-ATC ATT GGT CGC TGC CAA AGA-3') and semi-nested second round primers CPZ-GAG-F2 (5'-ATG ATG ACA GCT GTT CAA GGA GT-3') and CPZ-GAG-R1. PCR amplifications conditions included 45 cycles of denaturation

(94°C, 30 s), annealing (50°C, 30 s), and elongation (68°C, 1 min). Amplified products were directly sequenced. Sequences were deposited in Genbank (accession numbers AY542959 and AY542960).

Subspecies determination: To confirm the chimpanzee subspecies origin of faecal and urine samples from the various collection sites, a 450 bp region of the mtDNA genome (D loop) was amplified as described[1] using primers L15997 (5'-CACCATTAGCACCCAAAGCT-3') and H16498 (5'-CCTGAAGTAGGAACCAGATG-3'). The resulting products were sequenced directly. All samples were determined to be of *P. t. schweinfurthii* origin.

Phylogenetic analysis. The SIVcpzDRC1 partial gp41/*nef* and partial *gag* sequences were aligned manually against a panel of HIV-1 and SIVcpz reference sequences. Aside from GAB2 (F. Bibollet-Ruche and B. H. Hahn, unpublished data) these were all available in GenBank (accession numbers M62320, K02013, K03454, AJ006022, AJ271370, L20571, L20587, AF115393, AJ271369, AF103818, X52154, U42720, AF447763, AY181991, AY181992, and AY181993). The gp41/*nef* tree was outgroup-rooted with SIVgsn 99CM71 (AF468658) and the *gag* tree was rooted with SIVrcm Ng411 (AF349680). Maximum likelihood (ML) heuristic tree searches and bootstrapping (1000 replicates) were conducted on gapped-stripped alignments using PAUP*[4] under a general time-reversible (GTR) model with gamma-distributed rate heterogeneity among sites and SPR branch swapping. A Bayesian Markov Chain Monte Carlo analysis was done with MrBayes 3[5] under a GTR + G substitution model. Regardless of tree inference method, the best tree for each gene region placed SIVcpzDRC1 as the sister-taxon to the "TAN" lineage of SIVcpz (as depicted in Fig. 1d of the main text).

REFERENCES

1. Santiago, M. L. *et al.*, *J. Virol.* **77**, 7545-7562 (2003).
2. Lader, E. S. United States Patent Application 20010016312 (2001).
3. Santiago, M. L. *et al.*, *Science* **295**, 465 (2002).
4. Swofford, D. L. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts (2002).
5. Ronquist, F. and Huelsenbeck, J. P. *Bioinformatics* **19**, 1572-1574 (2003).