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COMMENTARY

Trunkloads of Viruses

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Elephant populations are under intense pressure internationally from habitat destruction and poaching for ivory and meat. They also face pressure from infectious agents, including elephant endotheliotropic herpesvirus 1 (EEHV1), which kills ~20% of Asian elephants (*Elephas maximus*) born in zoos and causes disease in the wild. EEHV1 is one of at least six distinct EEHV in a phylogenetic lineage that appears to represent an ancient but newly recognized subfamily (the *Deltaherpesvirinae*) in the family *Herpesviridae*.

Elephant endotheliotropic herpesvirus 1 (EEHV1) causes a rapidly progressing and usually fatal hemorrhagic disease that occurs in the wild in Asia and affects ~20% of Asian elephant (*Elephas maximus*) calves born in zoos in the United States and Europe (1). About 60% of juvenile deaths of captive elephants are attributed to such infections. Development of control measures has been hampered by the lack of systems for culture of the virus in laboratories. Its genetic study has been restricted to analysis of blood, trunk wash fluid, and tissue samples collected during necropsies. Fortunately, methods for amplifying DNA from uncharacterized viruses through the use of degenerate PCR primers that target highly conserved genes (2, 3), plus advances in DNA sequencing, have enabled assembly of complete genome sequences for EEHV1A and EEHV1B (4, 5), as well as numerous subgenomic segments from other EEHV present in clinical specimens. Among other things, this work has led to the discovery of what is now a dozen new herpesviruses of elephants, including five of the gammaherpesvirus lineage (6, 7). In two remarkable papers published in this issue of *Journal of Virology* (8, 9), L. K. Richman, J.-C. Zong, G. S. Hayward, and colleagues provide evidence to support recognition of five new EEHV species and show that these viruses and EEHV1 belong to a lineage within the family *Herpesviridae* that is distinct from its three currently recognized herpesvirus subfamilies (the *Alpha-*, *Beta-*, and *Gammaherpesvirinae*), the proposed *Deltaherpesvirinae*. This work provides a foundation for biological studies of the transmission and pathogenesis of these viruses and will be useful in developing the specific and informative diagnostic tools and therapeutic approaches needed to prevent these viruses from further devastating the already diminished international herd of these magnificent beasts.

A NEW HERPESVIRUS SUBFAMILY?

The current subfamilies within the *Herpesviridae* are harmonious with relationships readily deduced from genetic information, but they were established on the basis of shared biological properties more than 30 years ago, prior to the availability of biological sequences (10). Subsequent identification of viruses that share virion architecture but little genetic information with other herpesviruses led to the establishment in 2009 of two new virus families, the *Alloherpesviridae* (viruses of bony fish and frogs) and *Malacoherpesviridae* (viruses of oysters), and creation of the order *Herpesvirales* to encompass the three virus families (11) (Fig. 1A). The *Herpesvirales* stand as one of the seven virologic orders recognized by the International Committee for Taxonomy of Viruses (12).

To date, 88 virus species have been formally recognized within the *Herpesviridae* (the current complete list of approved virus taxons is available at http://ictvonline.org/). In addition, approximately 200 additional viruses detected using methods such as those described above await formal consideration (V. Lacoste, personal communication). With very few exceptions, the amino acid sequence of a small conserved segment of the viral DNA polymerase (~150 amino acids) is sufficient to not only reliably identify a virus as belonging to the evolutionary lineage represented by the *Herpesviridae*, but also their subfamily, and in most cases a recognized genus. Early analyses of such sequences from EEHV1 showed that sequences of its DNA polymerase and other highly conserved herpesvirus core genes branched near the base of the betaherpesvirus branch of the herpesvirus tree, leading to the suggestions that the virus might represent a new herpesvirus subfamily (13, 14). EEHV1 is currently formally recognized as the species *Elephantid herpesvirus 1*, the type species in the *Proboscivirus* genus within the *Betaherpesvirinae*. The new papers by Richman et al. (8) and Zong et al. (9) go well beyond this, showing that six distinct EEHV belong to the *Proboscivirus* lineage; the authors make the case that this lineage represents a novel subfamily within the *Herpesviridae*.

Evidence for recognition of a new subfamily comes in three main forms: (i) deep branching of conserved herpesvirus core sequences near the points of divergence of the beta- and gammaherpesvirus branches of phylogenetic trees (Fig. 1A); (ii) the presence of genes that are present in alphaherpesvirus and/or gammaherpesvirus genomes and are seldom if ever found in betaherpesviruses, as well as numerous genes that are unique to EEHV (~60 in the case of EEHV1, or half of its encoded proteins) (Fig. 2); and (iii) an arrangement of herpesvirus core gene blocks different from what has been seen in other herpesvirus lineages (Fig. 2). The latter is important because with few and limited exceptions, herpesvirus core genes are arranged in a colinear manner within subfamilies (15).

The question of whether the EEHV should be elevated to the level of a virus subfamily is under deliberation by the Herpesvi-

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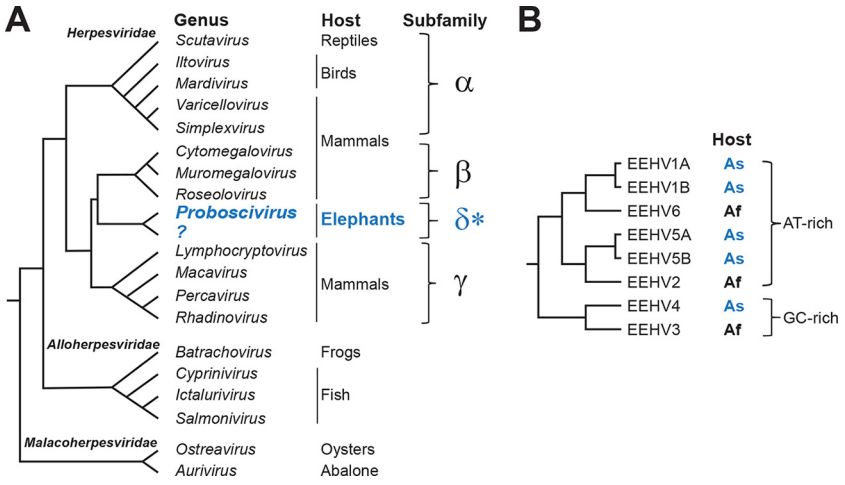

FIG 1 EEHV phylogeny and taxonomy. (A) EEHV lines of descent in the context of established herpesvirus taxonomy. With the exception of the EEHV, the taxonomic relationships shown are based on current herpesvirus taxonomy (http://ictvonline.org/). The proposed (indicated with a blue asterisk) *Deltaherpesvirinae* (δ) subfamily branches from between the *Betaherpesvirinae* (β) and *Gammaherpesvirinae* (γ) lineages. In constructing the diagram, it is assumed that the *Proboscivirus* genus would be retained in the proposed new subfamily, and the diagram indicates the possibility of establishing a new genus for the GC-rich viruses that are shown in panel B. (B) Relationships among the EEHV. AT- and GC-rich branches are shown, as well as the host species for each virus. The host species were Asian elephants (As) or African elephants (Af). This figure was adapted from reference 15.

rales Study Group of the International Committee for Taxonomy of Viruses. There are questions and competing arguments that go beyond the question of whether Greek letters will soon be in short supply. Will the other EEHV have genome organizations similar to that of EEHV1? Are there reasons to expect that ongoing searches for new viruses in other organisms will uncover a multitude of analogous new lineages? What unintended consequences might connect to recognition of a new lineage? What depiction of the relationships is most usefully informative? In any respect, the new data are clear that the lineage is deeply rooted within the *Herpesviridae*.

GENOMIC PLASTICITY AND DIVERSITY

Patterns of sequence variation are fundamental biological properties of individual viruses and groups of viruses. As a group, EEHV display forms and levels of sequence diversity that are collectively unique with respect to forms of variation seen for other groups of herpesviruses. Somewhat analogous to human herpesviruses 6A and 6B (16), most of the 64 genes that span the center of the EEHV1A and EEHV1B genomes are >99% identical, with three interspersed sharply bounded regions of much greater sequence divergence (referred to as chimeric domains, or CD), as well as some sequence rearrangements in the vicinity of the genomic termini (4, 8). Although less sequence is available, EEHV5A and EEHV5B appear to have a similar relationship (9). In contrast to human herpesvirus 6A (HHV-6A) and HHV-6B, the chimeric domains appear to be products of recombination with diverged versions of EEHV species that have not been detected and may no longer be extant. It remains to be determined whether either of these pairs of closely related EEHV represent distinct virus species.

Phylogenetically, the EEHV form two main clusters, one harboring two closely related pairs of viruses with relatively AT-rich genomes, and another cluster harboring EEHV3 and EEHV4, which have GC-rich genomes (Fig. 1B) (9). Each pair of closely related viruses includes one whose natural host appears to be Asian elephants, and one of African elephants. Thus, Asian and African elephants are both host to at least three distinct, but closely related herpesviruses of the EEHV lineage. A precedent is that humans are hosts to three alphaherpesviruses, herpes simplex viruses 1 and 2, and varicella-zoster virus (formally, *Human herpesviruses 1*, *2*, and *3*). Although there were early suggestions that

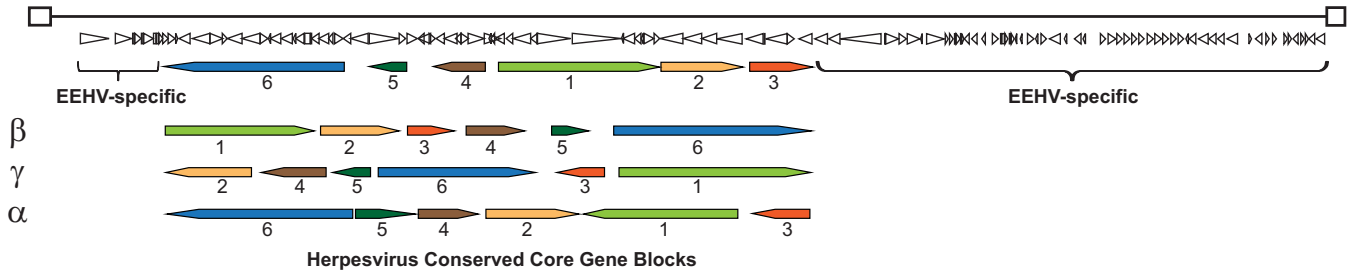

FIG 2 EEHV1 genomic architecture. The 180-kb genome consists of a unique segment bounded by a pair of direct terminal repeats. The locations of the six blocks of core genes conserved among members of the *Herpesviridae* are shown, along with the orientations of these regions characteristic of the alpha-, beta-, and gammaherpesvirus subfamilies. The conserved core region is flanked by genes not found in other herpesviruses. This figure is based on information in references 4, 8, 9, 12, and 15. The genomic orientation shown is reversed from the depictions in references 8 and 9.

severe hemorrhagic disease associated with EEHV1 was due to infection of Asian elephants with virus from African elephants (13), accumulated evidence shows that these viruses can cause disease as well as persist asymptomatically in their native hosts. While EEHV1 is thus far the major pathogen, the presence of multiple closely related host-specific viruses will complicate efforts to develop vaccines and therapeutic approaches.

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