JOURNAL OF GENERAL VIROLOGY

REVIEW

Jancarova *et al.*, *Journal of General Virology* 2023;104:001837

DOI 10.1099/jgv.0.001837

MICROBIOLOGY SOCIETY

OPEN ACCESS

The role of sand flies as vectors of viruses other than phleboviruses

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Abstract

Sand flies (Diptera: Phlebotominae) are proven vectors of various pathogens of medical and veterinary importance. Although mostly known for their pivotal role in the transmission of parasitic protists of the genus *Leishmania* that cause leishmaniases, they are also proven or suspected vectors of many arboviruses, some of which threaten human and animal health, causing disorders such as human encephalitis (Chandipura virus) or serious diseases of domestic animals (vesicular stomatitis viruses). We reviewed the literature to summarize the current published information on viruses detected in or isolated from phlebotomine sand flies, excluding the family *Phenuiviridae* with the genus *Phlebovirus*, as these have been well investigated and up-to-date reviews are available. Sand fly-borne viruses from four other families (*Rhabdoviridae*, *Flaviviridae*, *Reoviridae* and *Peribunyaviridae*) and one unclassified group (*Negevirus*) are reviewed for the first time regarding their distribution in nature, host and vector specificity, and potential natural transmission cycles.

INTRODUCTION

Phlebotomine sand flies (Diptera: Psychodidae) are regarded as important insects in human and veterinary medicine, as they are vectors of various pathogens that infect humans and both domestic and wild animals. Although primarily known as proven vectors of most disease-causing *Leishmania* species, they also transmit other pathogens, namely bacteria of the genus *Bartonella* and various viruses (reviewed in [1]). They are small insects with body size rarely exceeding 3 mm, typically densely covered by hairs, and their colour ranges from pale to dark shades of brown. Sand flies typically have a holometabolic life cycle (Fig. 1) that includes eggs, four larval instars, pupae and adults.

Unlike mosquitoes, their larval development occurs in terrestrial habitats, although humidity is one of the key factors, together with suitable temperature and other environmental requirements, that define their geographical distribution in vast regions of both the Old and New Worlds, roughly between latitudes 50 ° N and 40 ° S, with a notable absence in New Zealand and the

Received 21 November 2022; Accepted 08 March 2023; Published 05 April 2023

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Keywords: sand fly; sand fly-borne virus; *Vesiculovirus*; *Curiovirus*; *Sripuvirus*; *Arurhavirus*; *Flavivirus*; *Orbivirus*; *Pacuvirus*; *Orthobunyavirus*; arbovirus.

Abbreviations: *Ae.*, *Aedes*; ALMV, Almpiwar virus; *An.*, *Anopheles*; ANUV, Ananindeua virus; AP61, Mosquito tissue culture No. 61; ARUV, Aruac virus; BHK-21, Baby Hamster Kidney fibroblasts; C6/36, *Aedes albopictus* clone C6/36 cell line; C, capsid protein; CAIV, Caimito virus; CFAV, cell fusing agent virus; CGLV, Changuinola virus; CHIKV, Chikungunya virus; CHIV, Chilibre virus; CHPV, Chandipura virus; CHVV, Charleville virus; CJSV, Carajas virus; CNS, central nervous system; CPE, cytopathic effect; *Cx.*, *Culex*; dsRNA, double strand ribonucleic acid; E, envelope protein; ELISA, indirect enzyme-linked immunosorbent assay; ENM, Ecological Niche Modelling; EPEV, Ecuador Paraiso Escondido virus; F, filial generation; G, glycoprotein of Rhabdoviridae; Gc, external glycoprotein of Peribunyaviridae; GMAV, Guama virus; Gn, external glycoprotein of Peribunyaviridae; Hep-2, Human Epidermoid carcinoma #2 cell line; ICTV, International Committee on Taxonomy of Viruses; INHV, Inhangapi virus; ISFs, insect-specific flaviviruses; ISFV, Isfahan virus; L, large protein of Peribunyaviridae; L, polymerase of the Rhabdoviridae; LL-5, *Lutzomyia longipalpis*-5 cell line; *Lu.*, *Lutzomyia*; M, membrane protein; M, matrix protein of Rhabdoviridae; *M.*, *Melanoconion*; MARV, Maraba virus; MDCK, Madin-Darby canine kidney cells; MHV, Mahogany Hammock virus; MOJUV, Moju virus; MORV, Morreton virus; N, nucleocapsid protein of Rhabdoviridae; N, nucleocapsid protein of Peribunyaviridae; NCBI, National Center for Biotechnology Information; NIAV, Niakha virus; NKV, No Known Vector flaviviruses; NS1, non-structural protein 1; NS3, non-structural protein 3; NS5, non-structural protein 5; NS2a, non-structural protein 2a; NS4a, non-structural protein 4a; NS2b, non-structural protein 2b; NS4b, non-structural protein 4b; NSm, non-structural protein of Peribunyaviridae; NS1-NS4, non-structural Orbivirus proteins; NSs, non-structural protein of Peribunyaviridae; ORFs, open reading frames; P, phosphoprotein of Rhabdoviridae; PACV, Pacui virus; PCR, polymerase chane reaction; PERV, Perinet virus; PFU, plaque-forming unit; *Ph.*, *Phlebotomus*; p.i., post infection; PM, peritrophic matrix; PP-9, *Phlebotomus papatasi*-9 cell line; RADV, Radi virus; RdRP, RNA-directed RNA polymerase; RNA, ribonucleic acid; RPEV, Rio Preto da Eva virus; RT-PCR, reverse transcription polymerase chain reaction; SABV, Saboya virus; SBAV, Santa Barbara virus; *Se.*, *Sergentomyia*; SRIV, Sripur virus; -ssRNA, negative-sense single strand ribonucleic acid; STMV, Santarem virus; SW13, Scott and White No. 13 human cell line; TAPV, Tapirapé virus; VP1-VP7, structural orbivirus proteins; VSAV, vesicular stomatitis Alagoas virus; VSIV, vesicular stomatitis Indiana virus; VSNJV, vesicular stomatitis New Jersey virus; VSV, vesicular stomatitis virus; WHO, World Health Organization; XIBV, Xiburema virus; XTC-2, *Xenopus* Tadpole Carcass-2; YBV, Yug Bogdanovac.

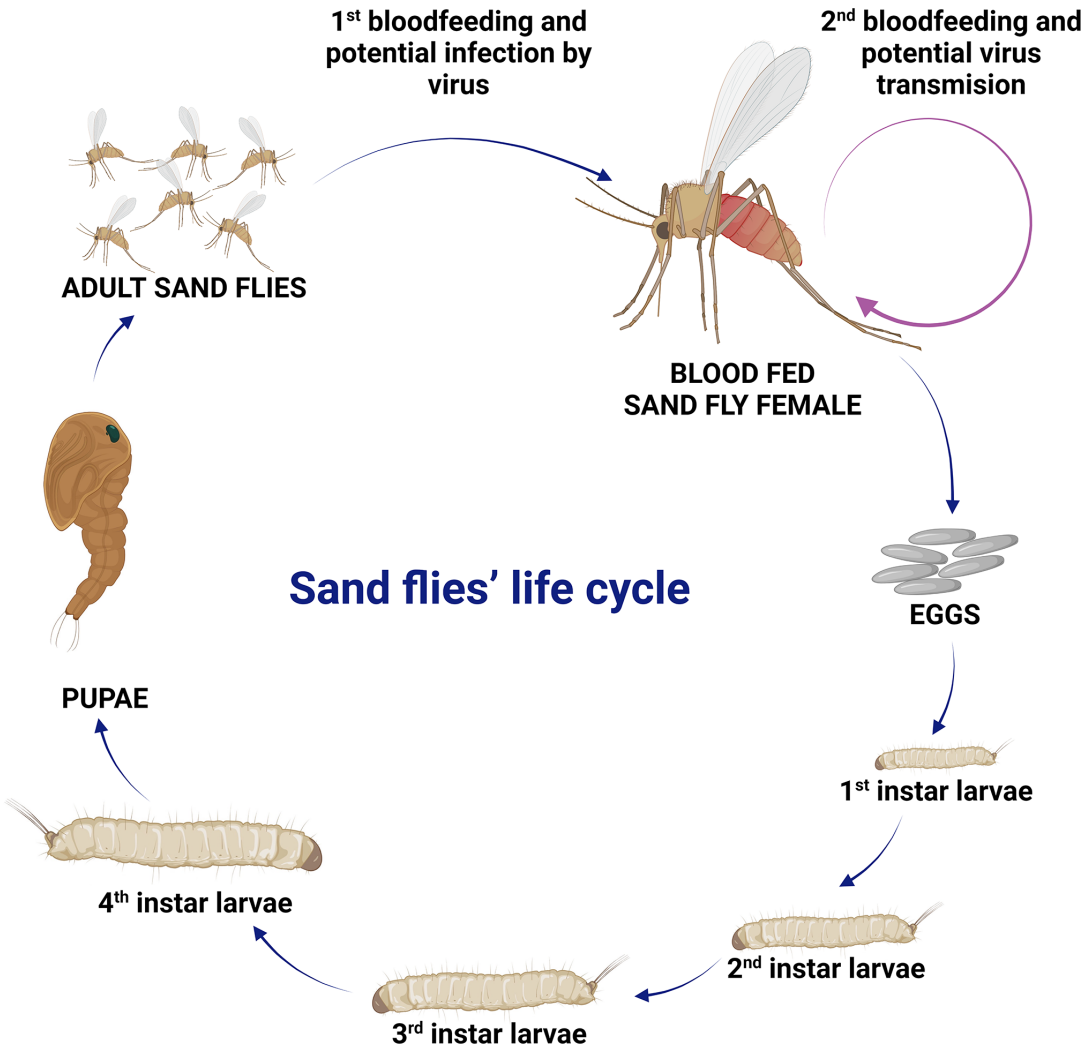

Fig. 1. Sand flies' life cycle. Sand fly females lay eggs about 1 week post-blood feeding. Larvae hatch from eggs and develop through four larval instars to the pupal stage. Adults emerging from pupa are ready to mate within a few days. The length of the entire life cycle depends on various factors, such as sand fly species or ambient temperature, and may vary from 2 months up to 1 year. During the first blood feeding, females are infected by the virus and they may transmit the virus to another host during the second blood feeding. Some viruses are also transmitted vertically to the progeny. (Created in BioRender.com.)

Pacific islands [2]. Their breeding sites and larval habitats are diverse and generally poorly defined but typically of rich organic content provided by potential hosts of the haematophagous adults (e.g. rodent burrows) [3]. Adults have nocturnal activity, hiding in dark places during the day. Both sexes feed on plant sap, nectar and honeydew, however, females of most species need to take blood meals on various vertebrate hosts in order to produce progeny after mating. Different degrees of association with humans, ranging from species occasionally biting people who accidentally enter their sylvatic habitats to those that breed close to human dwellings, are reflected by the varying epidemiological significance and involvement in the transmission of sand fly-borne pathogens among different species [1]. While Old World species tend to prefer arid and semiarid biotopes, New World sand flies are usually associated with a forest, although some species have adapted to other habitats, particularly in the drier areas, where they reside at peridomestic and urban sites [4].

Sand fly taxonomy and species identification, traditionally based on morphological characters, is now successfully combined with various molecular markers [4]. A conservative approach recognizes six main sand fly genera, three in the Old World (*Phlebotomus*, *Sergentomyia* and *Chinius*) and three in the New World (*Lutzomyia, Brumptomyia* and *Warileya*) [5]. In this review, albeit aware of a recently proposed revision of the New World genera that is expected to be followed by a similar rearrangement of the Old World genera and subgenera in the near future, we adhere to this simplified taxonomy outline. Therefore, for example, we refer to *Lutzomyia carrerai* as the vector of Santarem virus, even though this sand fly species is currently classified within the newly

elevated genus *Psychodopygus* according to the revised taxonomy. In the clinical perspective of medical entomology, species of only two genera, *Phlebotomus* in the Old World and *Lutzomyia* in the New World, are regarded as proven vectors of human and veterinary pathogens. However, growing evidence based on PCR positivity and direct microscopical observations of protozoan promastigotes also suggest a potential role of some species of the genus *Sergentomyia* in their transmission [6].

All sand fly-borne pathogens are expected to enter the insect body through the digestive tract. The sand fly gut consists of three major parts: foregut (stomodeum), midgut (mesentheron) and hindgut (proctodeum). The foregut (including the stomodeal valve) and the hindgut (including the pyloric triangle) are lined by chitin, while the midgut is composed of a single-layered epithelium with a brush border of microvilli. While a sugar meal enters first, the oesophageal diverticulum (crop), a bloodmeal is directed directly to the midgut. Within a few hours, the bloodmeal is surrounded by a peritrophic matrix (PM), an acellular layer composed of proteins, glycoproteins and chitin. It is secreted by the midgut epithelium in response to feeding and represents an important mechanical barrier to pathogens. At the end of the digestion process, the PM is degraded by sand fly chitinases [7], disintegrating on its posterior end [8]. The kinetics of PM synthesis and disintegration differs among sand fly species, in some (e.g. *Se. schwetzi*) breaking late, which results in defecation of ingested pathogens that were prevented from binding to the sand fly midgut epithelium [9].

To become established in the sand fly, viruses must infect the midgut epithelium and consequently other sand fly tissues and organs, including the salivary glands. However, compared to mosquitoes, barriers for virus development in sand flies are barely known. To complete the life cycle, viruses are co-inoculated into the vertebrate host together with sand fly saliva and midgut content. These components are proven to immunomodulate the host and there is a vast knowledge of the molecular mechanisms by which saliva affects mammalian immune cells (reviewed in [10, 11]). However, hosts repeatedly exposed to sand fly saliva or to uninfected sand flies develop delayed-type skin hypersensitivity, which creates an inhospitable environment for *Leishmania* injected by the vector [12, 13]. Currently, it is not known whether this effect is also important in virus transmission.

The World Health Organization (WHO) [14] established four main criteria to define a vector of a virus: (i) the virus must be isolated from wild-caught arthropods that contain no visible blood; (ii) arthropods become infected by feeding on viraemic vertebrate host or by artificial substitution; (iii) arthropods must be able to transmit the virus biologically by bite; and (iv) field evidence confirming association between arthropods and appropriate vertebrate host must exist. All these four criteria must be met by proven vectors. Isolation of the virus from insects caught in nature is not enough to define a vector as proven; in such a case, the sand fly species could be called 'suspected vector', indicating that only one WHO criterion had been satisfied. 'Potential vector' is one that has satisfied conditions about nature infection and experimental transmission [14].

The vectorial role of a sand fly species is determined by several aspects that include its feeding habits, population dynamics and susceptibility to pathogen infection. Current climate and environmental changes induced by various human activities are expected to facilitate the spread of sand flies and sand fly-borne pathogens; for example, deforestation has resulted in visceral leishmaniases becoming endemic in many areas of Amazonia [4]. Field studies on sand flies, combined with virus characterization and isolation, would contribute to a better understanding of vector–pathogen interaction and circulation in nature. The viruses detected so far in phlebotomine sand flies belong to five virus families, namely *Rhabdoviridae*, *Flaviviridae*, *Reoviridae*, *Peribunyaviridae* and *Phenuiviridae*. Among these, the family *Phenuiviridae* (*Bunyavirales*), which harbours the genus *Phlebovirus*, is by far the most studied and several recent reviews summarize our current knowledge of this family [15–19], whereas the remaining four families are far less known. Therefore, this review focuses on viruses within these four families and also one as yet unclassified group named *Negevirus*.

RHABDOVIRIDAE

The family *Rhabdoviridae* (*Mononegavirales*) comprises 45 genera that include 275 species [20, 21]. Their virion shape is bullet- or bacilliform-like, 45–100 nm in diameter and 100–430 nm in length. The genome of these viruses is negative-sense single-strand RNA (−ssRNA), which is either unsegmented or divided into two segments, with the length varying between 10 and 16 kb. The RNA genome typically contains genes that code five main proteins [nucleocapsid (N), phosphoprotein (P), matrix (M), glycoprotein (G) and polymerase (L)], even though members of some genera may possess additional genes that encode structural and non-structural proteins (reviewed in [22]).

The host range of rhabdoviruses is wide as they infect both plants and animals, including both invertebrates and vertebrates. Some of these viruses cause diseases of high medical and veterinary importance (reviewed in [23]). Members of four genera, namely *Vesiculovirus*, *Curiovirus*, *Sripuvirus* and *Arurhavirus*, were detected in phlebotomine sand flies and are discussed below.

Vesiculovirus (*Mononegavirales: Rhabdoviridae*)

The genus *Vesiculovirus* is monophyletic and includes 19 species [21] transmitted by insects and/or by direct contact, infecting various hosts among reptiles, birds and mammals. Bullet-shaped virions, with an approximate length of 190 nm and diameter of 85 nm, contain linear and single-stranded negative-sense RNA ~11 kb in size. In addition to leader and trailer sequences, their

Table 1. Vesiculoviruses isolated from phlebotomine sand flies

Virus species	Virus name	First isolated from sand fly species	First place of virus isolation (sand fly sampling year)	Reference
Vesiculovirus alagoas	Vesicular stomatitis Alagoas virus (VSAV)	*Lutzomyia* spp.	Colombia (1986)	[26]
Vesiculovirus indiana	Vesicular stomatitis Indiana virus (VSIV)	*Lutzomyia* spp.	Almirante, Panama, (1959–1962)	[42]
Vesiculovirus newjersey	Vesicular stomatitis New Jersey virus (VSNJV)	*Lu. shannoni*	Ossabaw Island, Georgia, USA (1988)	[40]
Vesiculovirus chandipura	Chandipura virus (CHPV)	*Phlebotomus* spp.	Aurangabad, Maharashtra State, India (1969)	[72]
Vesiculovirus isfahan	Isfahan virus (ISFV)	*Ph. papatasi*	Isfahan province, Iran (1975)	[84]
Vesiculovirus carajas	Carajas virus (CJSV)	*Lutzomyia* spp.	Maraba, Para State, Brazil (1983)	[86]
Vesiculovirus maraba	Maraba virus (MARV)	*Lutzomyia* spp.	Maraba, Para State, Brazil (1983)	[86]
Vesiculovirus bogdanovac	Yug Bogdanovac virus (YBV)	*Ph. perfiliewi*	Dobrić, Mačva District, Serbia (1976–1982)	[89]
Vesiculovirus morreton	Morreton virus (MORV)	*Lutzomyia* spp.	Durania, Colombia (1986)	[26]
Vesiculovirus perinet	Perinet virus (PERV)	*Ph. berentiensis*	Périnet, Madagascar (1978)	[191]
Vesiculovirus radi	Radi virus (RADV)	*Ph. perfiliewi*	Radi, Tuscany, Italy (1982)	[192]

*In the original publication, the authors referred to this species as *Sergentomyia berentiensis* [191].

genome codes five general structural proteins. Furthermore, some species possess additional open reading frames (ORFs) within the P gene that encode small basic proteins [22, 24]. Eleven *Vesiculovirus* species isolated from sand flies are listed in Table 1.

Vesicular stomatitis virus complex

Vesicular stomatitis viruses are endemic in the New World. However, disease outbreaks also occurred in Africa (1884, 1897, 1934, 1938 and 1943) and Europe during the First World War, probably due to horses transported from America [25]. These viruses cause stomatitis disease in ruminants, mainly in cattle, pigs and equids, but also rarely in humans. Their host range is probably wider; antibodies were also detected in raccoons, deer, dogs, pronghorns, peccaries, wild turkeys, monkeys, sloths, bobcats and porcupines [26–32], but the role of wild animals in virus circulation remains unclear.

Infected domestic animals develop lesions on their lips, gums, tongue, teats, prepuces and coronary band. The initial phase of this disease is often unrecognizable from foot-and-mouth disease. Vesicular stomatitis virus infection may be further complicated by secondary bacterial infection, but generally causes a rather short and self-limiting disease with a negligible mortality rate. However, the disease can cause significant economic losses by decreasing milk and meat production, and quarantine restrictions to control the disease spread have a serious impact on international trade with animals and their products [25, 33–36]. In humans, the disease may be subclinical or with clinical symptoms such as fever and acute flu-like illness, frequently accompanied by lesions on the tongue, oral mucosa, or pharynx. Although it usually passes over 3–6 days without any complications, rarely encephalitis can develop in children [25, 27, 37, 38].

While some uncertainties about VSV circulation still prevail, it is supposed that these viruses may be transmitted by direct contact, contaminated environment, or by insect vectors, either mechanically or biologically [25, 27, 37]. Sand flies are considered to be proven VSV vectors because they meet all given criteria for vector implication in several field and experimental studies [14]. This review focuses solely on sand flies and their role as VSV vectors; other vectors and ways of transmission have been summarized by Rozo-Lopez *et al.* [36].

Isolation of VSV from field-collected sand flies

In North America, VSNJV was obtained from pools of males and unfed females of *Lu. shannoni* surveyed in 2 consecutive years in Ossabaw Island, Georgia, USA. Four pools out of 2208 tested were positive for VSNJV. Relatively high virus titre in three pools (~4.5 $\log_{10}$ PFU) suggests virus replication in sand flies or the presence of several infected sand flies in the pool [39]. In the same area, VSNJV was obtained from 3 out of 186 pools of non-bloodfed females of *Lu. shannoni,* each pool containing 15–50 individuals [40]. Finally, VSAV was isolated from five pools of *Lutzomyia* spp. captured in 1986 in Colombia [26].

In Panama, VSIV was first detected in sand flies by Shelokov and Peralta [41] and Galindo *et al.* [42], and then between 1969 and 1971 a more detailed study was conducted by Tesh *et al.* [43]. They screened sand fly pools (50–100 females per pool; 50–250 males per pool) collected at various localities that differed by a predominant biotope, and they successfully isolated VSIV from 6 pools of females *Lutzomyia* sp. and from 3 pools of *Lu. trapidoi* females using Vero cells infection and intracerebral injection to suckling mice [43]. Repeated VSV isolations from field-caught males support the hypothesis of transovarial transmission [44, 45]. Although

there are no data suggesting sexual transmission of VSVs in sand flies, Rozo-Lopez *et al.* [46] described such transmission in biting midges, namely between VSV-infected females and naive males of *Culicoides sonorensis* and vice versa.

Experimental results: infections of sand flies with VSV

Experimental infections may be divided into two groups: (i) infection by oral feeding on viral suspension or on infected animals and (ii) infection through intrathoracic injection. The first approach better reflects natural conditions and could be used to prove vector competence. The second approach may provide a more standardized infection dose and a higher infection rate, but it is inappropriate to demonstrate vectorial competence; intrathoracic injections avoid passage through the peritrophic matrix and the midgut epithelium, which are suggested to be one of the important anti-virus barriers, especially in unnatural virus–vector combinations [47]. Therefore, intrathoracic injections may serve as a specific experimental model, but not as a confirmation of vector competence.

Lutzomyia trapidoi females fed on VSIV-infected hamster transmitted the virus to mice 3–5 days post-infection (p.i.). In addition, the observed significant growth of mean virus titres suggested VSIV replication in sand flies. Unfortunately, in this experimental design, the authors used field-caught sand fly specimens rather than a tested laboratory-reared colony and therefore the possibility that these sand flies had been infected with the virus already before the experiment cannot be ruled out [48].

More recently, *Lu. shannoni* females orally infected by VSNJV transmitted virus to mice on day 6 p.i. and females injected intrathoracically infected hamsters 3 days p.i [45]. Transmission electron microscopy of *Lu. shannoni* orally infected by VSNJV first revealed viral particles in the midgut epithelium 36 hours p.i., extensive replication within the midgut and fat body at 48–82 hours p.i. and colonization of salivary glands from day 5 p.i [49].

Lutzomyia longipalpis females intrathoracically injected by VSAV were able to transmit infection to newborn mice [26]. However, since the virus might not be able to overcome the midgut barrier under natural conditions and may be defecated, these experiments should be carefully interpreted.

Experimental results: transovarial VSV transmission

Lutzomyia trapidoi females infected through blood feeding on VSIV-positive hamsters transmitted the virus transovarially to the next generation with an efficiency of 21–25% in F1 adults. Females of this F1 generation were able to infect suckling hamsters by bite, with this showing that VSIV remained infectious. Descendants (F2 generation) were also VSIV-positive, suggesting efficient transovarial transmission that can maintain circulation in nature, even without the presence of suitable vertebrate hosts [44]. The authors also demonstrated that during transmission VSIV is localized inside the eggs and not on their chorion. Nevertheless, VSV transovarial transmission in sand flies seems to be species-specific because it was proven for *Lu. trapidoi* and *Lu. ylephiletrix* but not for *Lu. sanguinara* or *Lu. gomezi* [44]. In *Lu. shannoni*, the transovarial transmission seems to occur quite rarely; Comer *et al.* [45] described low efficiency of this type of transmission for VSNJV, ~1%, depending on the virus infectious dose, and Weaver *et al.* [49] did not find virus disseminating to ovaries on day 6 p.i. Four out of 88 *Lu. longipalpis* females infected intrathoracally by VSAV passed the virus transovarially to the F1 generation [26].

Potential VSV circulation in nature

Serological field studies proved VSV infection in humans, various domestic animals (cattle, pigs, horses, dogs) and wild animals (white-tailed deer, raccoons, feral swine, bobcats, opossums, porcupines, elks, mule deer, pronghorns, bighorn sheep, coyote, squirrels, rodents, wild turkey) [26, 27, 32]. However, experimental studies showed low viraemia that lasted only for a short time in the blood of deer mice and marmosets [50]. Newborn and young animals (vesper mice, marmosets, opossums, anteaters) seem to be more susceptible to infection, but they probably do not play an important role in VSV circulation [50]. By contrast, other animals (e.g. pigs, horses, feral swine, pronghorns, wood rats, raccoons, bats) that showed the presence of antibodies against VSV were negative for the presence of virus in the blood [32, 50–54].

Blood-fed females of *Lu. shannoni* captured on Ossabaw Island in Georgia, USA were analysed by indirect enzyme-linked immunosorbent assay (ELISA) to identify the most common vertebrates that serve as a blood source for this sand fly. The blood identified in most of the engorged sand fly females originates from white-tailed deer (*Odocoileus virginianus*) (81%) and feral swine (*Sus scrofa*) (16.2%) [55]. These animals also displayed VSV antibodies during field screenings in the same areas [51, 56]. However, VSV transmission from experimentally infected deer or pigs to *Lu. shannoni* was not detected [57, 58].

Only two studies focused on VSV transmission from the perspective of host viraemia. *Lutzomyia trapidoi* became infected after feeding on hamster with viraemia of at least $10^{4.5}$ PFU ml^{-1} blood, and all females that took blood on hamsters with lower viraemia ($10^{3.3}$–$10^{3.9}$ PFU ml^{-1} blood) were negative [48]. Experimental infections of *Lu. shannoni* by $10^{6.1}$ PFU and $10^{9.1}$ PFU of VSNJV ml^{-1} blood gave infection rates of 6.6 and 88.4%, respectively [45].

Interestingly, infected phlebotomine sand flies were also detected in the absence of clinical cases for domestic animals or humans, while other considered vectors such as mosquitoes, eye gnats, black flies, biting midges, house flies and other non-haematophagous

insects were only found to be infected during epidemics [25, 26]. Therefore, the source of such infections for sand flies needs to be further elucidated.

Susceptible animals with sufficiently high and long viraemia that have not yet been detected as reservoirs are expected to exist [48]. One potential candidate is the deer mouse *Peromyscus maniculatus*, where VSNJV in serum ranged between 7.5×10^2 and 7.5×10^4 PFU ml^{-1} during the first and the second day post-intranasal infection. In these 2 days, 12% of *Simulium vittatum* black flies fed on these nestlings became infected. Nevertheless, viraemia was not found in older mice and the virus was not detected in juveniles at 1, 2, 3, 10 or 11 days p.i [54]. However, as mentioned above, the role of newborns in virus maintenance in nature remains speculative. Mead *et al.* [59] fed black flies inoculated intrathoracally by VSVNJ on *Peromyscus maniculatus* and then exposed these deer mice to naive black fly females 24 and 48 hours p.i. Black fly females were infected by the virus, while no viraemia was detected in the mice themselves. This suggests that vertebrate viraemia screening may not be sufficient for reservoir determination, and more detailed studies are needed to clarify this phenomenon [59].

As indicated by the above described data, there are still some uncertainties regarding the circulation of VSVs between haematophagous vectors and vertebrates as well as the maintenance of the virus between outbreaks, suggesting that these processes may be more complex and not restricted solely to circulation between haematophagous insects and viraemic vertebrates, but rather utilizing several pathways that may act synergistically and remain to be elucidated.

Vesiculovirus chandipura

Chandipura virus (CHPV) is one of the most important causative agents of acute encephalitis syndrome outbreaks in India. It affects the central nervous system (CNS), mainly in children under the age of 15 years, and its symptoms are characterized by a rapid onset of fever, accompanied by arthralgia, altered sensorium, convulsions and serious complications that can be fatal [60–62]. Outside of India, CHPV was detected in West Africa (Senegal, Nigeria) [63–65] and Sri Lanka [66], although information about its occurrence is quite fragmentary and potentially outdated.

CHPV was isolated from humans [60, 64, 67] and hedgehog *Atelerix albiventris* [63] and a serological survey proved anti-CHPV antibodies in humans [60, 67, 68], toque macaques (*Macaca sinica*) [66] and domestic animals such as pigs, buffalo, cattle, goats, sheep and dogs [69]. The clinical signs of CHVP were only shown by experimentally infected domestic animals (ponies, oxen, goats) that developed ulcer at the inoculation site; however, no other symptoms were observed and reisolations of the virus from tissues other than the inoculation site were unsuccessful [70]. However, information about the disease and its progression in animals under natural conditions is lacking.

Sand flies are presumed to be CHPV vectors, although this claim has not yet been fully confirmed. The RNA of the virus was detected in a 'pool' of *Sergentomyia* sp. containing only two specimens [71] and in another pool of other unidentified sand flies [67], both collected during encephalitis outbreaks at the surveyed catching sites in India. Rao *et al.* [60] found CHPV RNA in a pool of sand flies collected in the house of a patient affected during the outbreak. CHPV was first isolated from a pool of 253 individuals collected in India; the isolated virus killed infant mice and caused a quick cytopathic effect on BHK-21 cells [72]. More recently, Sudeep *et al.* [73] isolated CHPV using Vero cells from a pool of *Sergentomyia* spp. that included only two females. Outside of India, the virus was repeatedly isolated from sand fly pools in Senegal [64, 65]; the virus isolate infected cell culture, caused a cytopathic effect and killed newborn mice within 1–2 days [64].

Transmission experiments were performed using various CHPV–sand fly models. In *Ph. papatasi* intrathoracically inoculated by CHPV, the mean of virus titres increased rapidly during the first 24 h, suggesting replication of the virus, but then decreased slightly. More importantly, infected *Ph. papatasi* females transmitted the virus by bite to sucking mice (on day 7 post-injection) and also transovarially to the F1 generation [74]. The authors also claimed that *Ph. papatasi* infected by feeding on viraemic mice transmit the infection to naive mice 4 and 7 days p.i., but these data were not published [74]. Other authors described the transmission of CHPV from intrathoracically injected *Ph. papatasi* males to females by mating [75].

In *Ph. argentipes*, a species commonly found in India, oral infection of females by CHPV led to virus dissemination to the head of sand flies and intrathoracically infected females transmitted the virus by bite to mice [76]. However, the sand flies used for these experiments originated from F1 adults of sand flies trapped in the field. Considering that transovarial transmission of CHPV has been repeatedly suggested [77, 78], this may affect the results of such an experimental design.

Tissue culture infections showed that CHPV can infect both invertebrate cells (LL-5, PP-9, *Aedes aegypti*; *Anopheles gambiae*; *Ae. malayensis*; *Ae. pseudoscutellaris*; *An. stephensi*) and vertebrate cells (MDCK, Vero, rhabdomyosarcoma, NIV-BtEPC, XTC-2 and porcine stable kidney). Interestingly, vertebrate cell lines showed cytopathic effect (CPE) quite early p.i., while no CPE was observed in insect cell lines [60, 79–83].

Chandipura virus detection in naturally infected sand flies [67, 71, 73], its oral infection [76] and the fact that injected sand flies transmitted CHPV sexually, transovarially and by bite [74, 76, 78] all suggest that sand flies are the main CHPV vectors. Nevertheless, the hypotheses about CHPV life cycle and transmission still await conclusive experimental confirmation.

Vesiculovirus isfahan

Isfahan virus (ISFV) was firstly isolated in 1975 in Isfahan province, Iran, from 2 pools (25 and 37 females) of *Ph. papatasi*. It caused a CPE after Vero cells infection and killed newborn mice within 24 h after intracerebral injection with 10^5 PFU ml^{-1}. The screening of anti-Isfahan antibodies in blood sera from humans and various animals was performed in the same region. Surprisingly, only humans and gerbils (*Rhombomys opinus*) were positive, in contrast to domestic animals, which were shown to serve as a dominant source of blood for *Ph. papatasi* [84]. Another successful isolation of Isfahan virus was from two pools of engorged *Ph. papatasi* females caught in gerbil colonies in Turkmenistan [85].

Experimentally infected domestic animals (ponies, oxen, goats, sheep and pigs) did not develop any signs of infection, except two ponies where lesions occurred at the inoculation site and healed 8 days p.i.; Isfahan virus was not detected in blood, mucus or any tissue obtained during necropsy at the end of the experiment. In laboratory animals, the outcome of infection was similar to that for VSV: newborn mice or hamsters died after infection while adults survived [70]. In culture, ISFV inoculation infected the LL5 cells and replicated without CPE, even though with a lower rate than other vesiculoviruses (VSIV and CHPV) [80]. Available data suggest that, compared to VSV, Isfahan virus causes milder disease in humans and domestic animals and mainly circulates in gerbil colonies.

Vesiculovirus carajas

This vesiculovirus was first isolated from a single pool of 100 males and 2 pools of females of unidentified *Lutzomyia* spp. caught in Brazil [86]. An isolated virus was used for *Lu. longipalpis* infections. After intrathoracic injection, the virus replicated in sand flies, the mean virus titre growing from 1.7 $\log_{10}$ PFU per individual at D0 post-inoculation to 4.6 $\log_{10}$ PFU per individual D7 post-inoculation. Infected females also transmitted the infection to their offspring; the progeny infection rate was 3.4% (5/109). After oral infection through blood feeding, no virus was found in females from the third day p.i. These results suggest that *Lu. longipalpis* is not a susceptible vector of Carajas virus, which can also explain the low percentage of successful transovarial transmission after intrathoracic injection [86].

Vesiculovirus maraba

Maraba virus was isolated from a pool containing 70 *Lutzomyia* spp. females caught in the same area as Carajas virus. It also appeared to replicate in *Lu. longipalpis* after intrathoracic infection and was transmitted to the progeny. However, oral sand fly oral infection was not tested [86]. The effect of Maraba virus infection was tested on both suckling (2-day-old) [87] and adult mice [88]. In both studies, the animals were infected by the Maraba virus intranasally and the virus showed high generalized neurotropism with high mortality of experimental animals (more than 25 and 100% for suckling mice and adult mice, respectively) [87, 88].

Vesiculovirus bogdanovac

This vesiculovirus was isolated from the *Ph. perfiliewi* pool containing 200 non-engorged females caught around Dobrić village in south-east Serbia. The supernatant of the pooled sample was inoculated intraperitoneally and intracerebrally into suckling mice that showed disease symptoms 7–8 days p.i. (nevertheless, the authors did not specify the symptoms and duration of the disease) [89]. The presence of this virus in the Dobrić area was also supported by a serological study. Among the sera of 274 humans and 54 domestic animals, complement-fixing antibodies were found in six and seven samples, respectively [89]. However, it is not yet known whether this vesiculovirus causes disease in humans or domestic animals.

Curiovirus (*Mononegavirales: Rhabdoviridae*)

The genus *Curiovirus* comprises four virus species that form a monophyletic group [24, 90]. They were isolated from various haematophagous insects (biting midges, sand flies, or mosquitoes) and all were detected in South America and the West Indies [91]. Their bullet-shaped virions contain negative-sense ssRNA with a size range of 12.6–13.6 kb, which codes five *Rhabdoviridae* general genes (*N*, *P*, *M*, *G* and *L*) and multiple additional ORFs between them [24].

Iriri virus (*Curiovirus iriri*) is the only species isolated from sand flies, namely unidentified *Lutzomyia* spp. collected in 1982 in Altamira, Para, Brazil [91]. However, in addition to the original study, there are no more data on this species. Similarly, little is known about the other three species of the genus detected in other haematophagous dipterans. Rochambeau virus (*Curiovirus rochambeau*) was isolated from mosquito (*Coquillettidia albicosta*) in 1973 by J.P. Digoutte and P. Fauran in French Guiana [92] and from grey kingbird (*Tyrannus dominicensis*) [93]. Diniz *et al.* [94, 95] studied Curionopolis virus (*Curiovirus curionopolis*) and Itacaiunas virus (*Curiovirus itacaiunas*) isolated from biting midges and demonstrated that both viruses infected newborn mice after intracerebral and intranasal inoculation. Itacaiunas virus infected mosquito cell lines (C6/36), without causing any CPE. Neither of these viruses infected mammals' Vero, RD or Hep-2 cells but anti-curionopolis virus antibodies were detected in coati (*Nasua nasua*) and a nonhuman primate tufted capuchin (*Cebus apella*), implying that this virus may infect mammals [94, 95]. In addition to the isolation of the Iriri virus from unidentified *Lutzomyia* sp. sand flies, there are no further data supporting the role of sand flies as proven vectors of other *Curiovirus* species, and the whole genus remains much understudied.

Sripuvirus (*Mononegavirales: Rhabdoviridae*)

According to the International Committee on Taxonomy of Viruses (ICTV), the genus *Sripuvirus* currently contains eight species [Almpiwar virus (*Sripuvirus almpiwar*), Chaco virus (*Sripuvirus chaco*), Charleville virus (*Sripuvirus charleville*), Cuiaba virus (*Sripuvirus cuiaba*), Hainan black-spectacled toad rhabdovirus (*Sripuvirus hainan*), Sena Madureira virus (*Spripuvirus madureira*), Niakha virus (*Sripuvirus niakha*) and Sripur virus (*Sripuvirus sripur*)], which form a monophyletic group [90, 96] and, in addition to these, two unclassified viruses (Timbo virus and Humpty Doo virus) also exist [21]. Sripuviruses possess a single molecule of negative-sense ssRNA with a size of ~11.0–11.5 kb that includes five typical *Rhabdoviridae* genes (*N*, *P*, *M*, *G*, *L*) and multiple additional ORFs [24, 96].

Most sripuviruses were only isolated from vertebrates, namely toads or lizards [97, 98]. However, three species of the genus *Sripuvirus* were isolated from sand flies: Niakha virus (NIAV), Charleville virus (CHVV) and Sripur virus (SRIV) [64, 97, 99, 100], and it seems that reptiles and sand flies are involved in circulation of these viruses in nature [97, 100, 101].

Niakha virus was first isolated from a mixed pool of 200 sand flies (*Ph. duboscqi* and *Sergentomyia* sp.) collected in Niakha, Senegal in 1992 [64] and later characterized by Vasilakis *et al.* [100]. This virus infected mammal BHK, Vero E6, mosquitoes C6/36 and sand fly PP-9 cells, producing CPE in all except the PP-9 cells. Vertebrate infections were only tested in mice; NIAV killed newborns, 3 days p.i. when infected intracerebrally, and 7 days p.i. when infected intraperitoneally or subcutaneously [64, 100]. So far, there is no evidence that NIAV infection causes disease in domestic animals or humans.

Charleville virus was isolated from unidentified *Phlebotomus* sp. sand flies caught in 1969 at Charleville in south-western Queensland, Australia. Other successful isolations of this virus were from a gecko *Gehyra australis* and once it was detected in *Forcipomyia* (*Lasiohelia*) sp. midges at the Mitchell River Aboriginal community located on the western shore of the Gulf of Carpentaria in far northern Queensland, Australia [99]. Sripur virus was isolated from the *Sergentomyia* sp. pool collected in 1973 in West Bengal, India [97].

Other sripuviruses isolated from lizards have so far not been found in sand flies but are known to replicate in mosquitoes. Almpiwar virus (ALMV) was repeatedly isolated from a skink *Cryptoblepharus pulcher* in northern Queensland, Australia [97, 102]. This virus species has never been isolated from invertebrates, but Carley *et al.* [103] showed virus multiplication after successful experimental infections of *Cx. fatigans* mosquitoes. Furthermore, phylogenetic analyses showed that ALMV is closely related to NIAV virus isolated from sand flies [64, 102]. Chaco virus isolated from lizards *Ameiva ameiva* and *Kentropyx calcarata* collected in 1962–1963 in Brazil killed newborn mice after intracranial but not after intraperitoneal inoculation. It was also inoculated into *Ae. aegypti*, where it was successfully multiplied in the salivary glands and transovarially transmitted to the next eight generations. In the same study, Timbo virus, closely related to *Sripuvirus*, was also isolated from the lizard *Ameiva ameiva* [101]. The second closely related virus, Humpty Doo virus, was isolated from pools of *Culicoides marksi* and *Lasiohelea* spp. in Australia [104]. However, these two last named viruses have not yet been classified as members of the genus *Sripuvirus* [21].

Arurhavirus (*Mononegavirales: Rhabdoviridae*)

The genus *Arurhavirus* was established only recently and so far includes four species [90]. Virions of species within this genus are similar to other members of the large family *Rhabdoviridae*, their RNA contains genes for five typical proteins and one or two additional genes are present in their genome [24, 105, 106]. Although it belongs to the well-studied family *Rhabdoviridae*, the knowledge of this newly characterized genus is still incomplete and more isolations from both hosts and vectors will be needed to better understand their biology.

The first *Arurhavirus* species isolated from sand flies was Inhangapi virus (*Arurhavirus inhangapi*, INHV), isolated from a pool of 109 unengorged females of *Lu. flaviscutellata* in Catu Forest, Pará State, Brazil in 1969 [107]. Based on virus isolation and sero-neutralization or complement fixation data, Walker *et al.* [24] suggested that rodents *Proechimys guyannensis*, *Hylaeamys megacephalus* and *Coendou* sp. can be infected by INHV. The nearly complete sequence of the INHV genome was published by Wanzeller *et al.* [106]. Santa Barbara virus (*Arurhavirus santabarbara*, SBAV) was isolated from unspecified mice caught in Santa Bárbara do Pará, Pará State, Brazil [90]. Although this was initially considered to be the first isolation of this virus, by searching National Center for Biotechnology Information (NCBI) GenBank we revealed that the same virus (strain AR775619, NCBI GenBank reference sequence: NC_028234.1) had previously been isolated from an unspecified species of the family *Psychodidae* in Pará state, Brazil, in 2010.

The last two members of Arurhavirus were isolated from mosquitoes. Aruac virus (*Arurhavirus aruac*, ARUV) was isolated from a pool of 32 *Trichoprosopon theobaldi* mosquitoes collected in the 1955 in Melaju Forest, Trinidad and Tobago [108]. The virus seems to have a low pathogenicity for mammals, as it only kills suckling mice after intracerebral injection. No natural link between ARUV and any vertebrate (humans, monkeys, birds, rodents) has so far been proven [108]. The second mosquito arurhavirus is Xiburema virus (*Arurhavirus xiburema*, XIBV), isolated from *Sabethes intermedius* sampled in Sena Madureira, Acre State, Brazil [97].

FLAVIVIRUS (*AMARILLOVIRALES: FLAVIVIRIDAE*)

Flaviviruses represent a heterogeneous group with a widespread geographical distribution. According to the ICTV, the genus *Flavivirus* currently comprises 53 species, including viruses pathogenic to vertebrates and those strictly bound to insects. Flaviviruses are small, enveloped viruses, with a virion size of 40–60 nm, containing a positive-sense and single-stranded RNA genome with a size of approximately 9.2–11.0 kb. There is usually a single ORF encoding polyprotein that is processed by proteases on three structural proteins (C-capsid, M-membrane, E-envelope) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5). Although flaviviruses share similar genomic organizations, their particular life cycles, host specificity and transmission modes differ significantly [109].

Flaviviruses can be divided into three main groups [110].

(i) Dual-host flaviviruses transmitted between haematophagous arthropods (ticks/mosquitoes) and vertebrate hosts.

(ii) Vertebrate-specific flaviviruses, also called no known vector (NKV), are currently subdivided into bat- and rodent-associated NKV flaviviruses. They are potentially transmitted by unidentified invertebrate hosts and/or by saliva or aerosol between hosts.

(iii) Insect-specific flaviviruses (ISFs) are unable to replicate in vertebrates; they are subdivided into classical ISF (monophyletic) and dual ISF (paraphyletic) groups.

Dual-host flaviviruses detected in sand flies

Flaviviruses are rarely detected in sand flies. Four strains of Saboya virus (*Saboya virus*, SABV) were isolated from polyspecific sand fly pools caught in Senegal in 1992. This virus did not grow on Vero or AP61 cells (from *Ae. pseudoscutedlaris*), but injection of sand fly homogenate into suckling mice led to virus isolation [64]. The appearance of SABV in sand flies in this area was later confirmed by Traoré-Lamizana *et al.* [65]. However, SABV vector specificity to sand flies is unclear; other authors obtained this virus from *Aedes* mosquitoes [64]. Moreover, Butenko [111] also detected SABV in *Ixodidae* ticks. According to phylogenetic analysis, SABV belongs to a dual-host group of flaviviruses that circulate between mosquitoes and vertebrates [110]. This corresponds to the repeated isolation of this virus from Kemp's gerbils (*Tatera kempi*), Gambian slit-faced bats (*Nycteris gambiensis)* and fiscal shrike birds (*Lanius collaris*) [111, 112]. Furthermore, anti-SABV antibodies were detected in closely unspecified animal species belonging to insectivores, primates, rodents, carnivores, even-toed ungulates, reptiles and birds [113]. However, confirmation of the expected SABV life cycle by laboratory studies is needed.

A single flavivirus was described from sand flies in the New World [114]. The virus, named Ecuador Paraiso Escondido virus (EPEV), was isolated from the pool of 30 non-engorged and non-gravid *Lu.* (*Psathyromyia*) *abonnenci* females. The replication of this virus in C6/36 cells was confirmed by visible CPE and RT-PCR. However, this virus did not infect or replicate in vertebrate cells (human SW13, hamster BHK, monkey Vero, amphibian XTC) or in suckling mice [114]. Ecuador Paraiso Escondido virus is a candidate for a new virus species; nevertheless, its phylogenetic position is surprising. Although replication solely in insect cells suggests its high similarity to the insect-specific group, phylogenetic analysis showed that EPEV appears to be more related to the common ancestral lineage of dual host flaviviruses circulating horizontally in mosquitoes–vertebrates and to vertebrate-specific flaviviruses (NKV) [114].

Insect-specific flaviviruses

Viruses belonging to the insect-specific flavivirus group represent part of the insect virome. ISFs cannot infect vertebrates or vertebrate tissue and cells, and they have been isolated from various mosquitoes collected in the Americas, Europe, Africa, Australia and Asia [110]. Some authors suggest that these viruses can potentially be used, either as a wild type or modified virus, to control medically important pathogens transmitted by haematophagous insects [115]. Superinfections by ISFs have already been shown to have an impact on the reduction of viral loads of vertebrate pathogenic flaviviruses (e.g. West Nile virus, dengue virus, Zika virus, etc.) [116–121]. However, until now, only a few viral sequences corresponding to ISF have been obtained from sand flies and virus isolation experiments have not been attempted.

The RNA sequences of two flaviviruses were found by Moureau *et al.* [122] in two pools of sand flies collected in Algeria, each containing 30 *Ph. perniciosus* males. The sequences seem to be most related to insect-specific flaviviruses, mainly the ISF group hosted by *Culex*, but the authors were not able to isolate viruses [122].

Another *Flavivirus* with a sequence related to insect-specific viruses was isolated in Portugal; unfortunately, the sequence data were only submitted to the NCBI GenBank database (with the name 'Flavivirus Phlebotomine/76/Arrabida/2007', NCBI GenBank reference sequence: HM563684.1) without any detailed information on the sand fly species of origin.

The fragment of the NS5 gene belonging to another recently described flavivirus, provisionally named Drežnica virus, was detected in pooled sand flies caught in Bosnia and Herzegovina. Phylogenetic analyses revealed that this virus clusters together with the sequence of flavivirus found in the genome of *Culiseta annulata*. Unfortunately, the attempt to isolate this virus was not successful

and the authors did not provide information about the species of sand flies included in the tested pools, despite mentioning that species identification had been performed [123].

Further flavivirus sequences were detected in again unspecified sand flies collected in Spain. These sequences are related to cell fusing agent virus (CFAV) isolated from various mosquitoes and *Culex* flavivirus Tokyo strain [124].

The number of insect-specific flaviviruses detected in sand flies is apparently smaller than of those detected in mosquitoes [125]. This phenomenon is probably biased as mosquitoes are generally studied more intensively due to their importance for human health. Screening for new sand fly ISFs is also neglected as the primary focus of field studies seeking to detect sand fly-borne viruses is mostly targeted on phleboviruses or flaviviruses that infect vertebrates. Therefore, the search of available sand fly genomes for the presence of endogenous viral elements as the well as the focus on isolation experiments would contribute towards an improved understanding of this particular group of viruses.

ORBIVIRUS (*REOVIRALES: SEDOREOVIRIDAE*)

Orbiviruses are transmitted by insects and ticks; for some, vectors remain unknown. Virions with a size of 65–80 nm with icosahedral shape contain 10 segments of dsRNA that encode structural (VP1–VP7) and non-structural (NS1–NS4) proteins. Most of the dsRNA segments contain a single major ORF, with the only two exceptions being genes for seg9 and a novel protein coded by two unique but overlapping ORFs [126–130]. Phylogenetic analyses divided species of this genus into three groups that correspond to their vectors: *Culicoides*-, mosquito- and tick-borne orbiviruses. The only sand fly-borne orbivirus, Changuinola virus (*Changuinola virus*, CGLV), has so far been described, clustered together with the *Culicoides*-borne group [129, 131].

To date, there are 12 described serotypes of the *Changuinola virus* species: Almeirim virus, Altamira virus, Canindé virus, Changuinola virus, Gurupi virus, Irituia virus, Jamanxi virus, Jari virus, Monte Dourado virus, Ourém virus, Purus virus and Saracá virus, but more recently unidentified serotypes are expected to exist [132]. The presence of this virus was recorded in Panama, Costa Rica, and tropical regions of South America and recently also in Asia – Thailand [43, 131, 133–135]. Sand flies are considered to be putative CGVL vectors, but experimental data to support this hypothesis are still missing. The first successful isolation of this virus (strain BT-436) was carried out during an entomological survey in Panama of unspecified pooled sand flies and also of human sera subsequently inoculated in suckling mice [42]. Similarly, CGVL (strain BT-436) was also isolated from unspecified sand flies by Peralta and Shelokov [136] in Panama 1959–1961. During the following field study in Panama, CGVL was repeatedly isolated by Vero cell infection and newborn mice intracerebral inoculation from 56 pools containing females, and 1 pool of male *Lutzomyia* sp., 70 pools of *Lu. trapidoi* females and 12 pools of *Lu. ylephilator* females. It was constantly present in sand flies during almost all tested periods 1969–1971 [43]. These viruses were shown to replicate in LL-5 cells, where they caused CPE [80, 133]. In 2019, CGVL was also detected in sand flies caught in Thailand (Trang province). The RNA of this virus was obtained from two female *Idiophlebotomus* sp., two *Se. khawi* and three *Ph. papatasi*. Phylogenetic analysis based on the VP1 region revealed that these Thai CGVL sequences are closely related to isolates from Brazil and Panama, *Lutzomyia* spp. and *Lu. panamensis*, respectively [135].

Changuinola virus is expected to circulate between sand flies and sloths, from which the virus was repeatedly isolated and confirmed serologically [134, 137]. Sloths, which are known to be a frequent source of blood for sand flies [138, 139], also displayed long-term CGLV viraemia that lasted up to 1 month [137]. However, this proposed circulation may not fully explain the transmission cycle, and other, yet unrecognized, CGVL vectors and competent hosts may be incriminated. In addition to sand fly cell culture, CGVL grows in mosquito cells (C6/36), *C. sonorensis* (KC) and mammalian (Vero) cells [43, 130, 133, 140]. Other viruses from this group were also isolated from mosquitoes, rice rats (*Hylaeamys megacephalus*), nine-banded armadillos (*Dasypus novemcinctus*), Linnaeus's two-toed sloths (*Choloepus didactylus*) and humans [42, 133, 141], and anti-CGVL antibodies were also detected in primates (*Alouatta pigra*, *Aotus trivirgatus*) [137]. CGVL pathogenicity for vertebrates is still questionable. Intracerebral inoculation of newborn mice and hamsters is fatal, but natural infection of humans is probably very rare or asymptomatic; only a single symptomatic human case has been reported, a CGVL isolation from the blood of a febrile entomologist in Panama in 1966 [43, 130, 133]. From this perspective, CGVL is currently not considered a significant human pathogen [140].

PERIBUNYAVIRIDAE

The ICVT distinguishes 7 genera with 115 species within this family [21]. Peribunyavirus virions are spherical or pleomorphic in shape and 80–120 nm in diameter and contain three segments (S, M and L) of a negative-sense ssRNA with a length of 11.2–12.5 kb in total. It contains genes for two external glycoproteins (Gn and Gc), a nucleocapsid protein (N) and a large protein (L) that possesses RNA-directed RNA polymerase (RdRP) and endonuclease functions; besides these, some viruses also code non-structural proteins (NSs and NSm) [142, 143].

Members of the family *Peribunyaviridae* have a broad range of hosts, some of them infecting both vertebrates and invertebrates, and others that have been found to be arthropod-specific (reviewed in [144]). Among the four genera within this family, viruses found in phlebotomine sand flies are represented in two.

Pacuvirus (*Bunyavirales: Peribunyaviridae*)

The genus *Pacuvirus* contains five virus species: *Caimito pacuvirus* (Caimito virus, CAIV), *Chilibre pacuvirus* (Chilibre virus, CHIV), *Pacui pacuvirus* (Pacui virus, PACV), *Rio Preto da Eva pacuvirus* (Rio Preto da Eva virus, RPEV) and *Tapirape pacuvirus* (Tapirapé virus, TAPV), and one related, unclassified virus, Santarem virus (STMV). Pacuviruses have been isolated from sand flies and rodents in Brazil and Trinidad, suggesting that natural circulation occurs through vertebrate–arthropod transmission cycles [144–147].

Pacui virus was originally classified as phlebovirus due to its connection with sand flies; however, later analyses revealed a close relationship of pacuviruses with orthobunyaviruses and placed them in *Peribunyaviridae* [144, 145, 147]. This virus was first isolated in 1961 from the serum of two rice rats (*Hylaeamys megacephalus*), the first caught in the Brazilian state Para [148] and the second in Bush Bush Forest in Trinidad and Tobago, and a cane rat (*Zygodontomys brevicauda*) caught in Bush Bush Forest as well [146]. Later in 1968, Pacui virus was isolated from pools of engorged and unengorged females and males of *Lu. flaviscutellata* trapped in Brazil, suggesting that transovarial and/or sexual transmission is possible. This sand fly species may be a specific vector as attempts to isolate the virus from *Lu. infraspinosa*, the second most abundant local species, as well as from large pools of local mosquitoes, were unsuccessful [107]. The virus was also isolated from rice rats (*Hylaeamys megacephalus*) by the same team and anti-PACV antibodies were detected in various rodents and marsupials but not in 464 humans living or working in the study area [107]. In experimental assays, Pacui virus infected LL-5 cells, but did not replicate or cause CPE [80].

Caimito virus, isolate VP-488A, was isolated from a sample of pooled females of *Lu. ylephilator*, collected in 1971 in El Aguacate, Panama, and showed low pathogenicity for infant mice [43]. Chilibre virus (referring to isolate VP-118D, CHIV) was first isolated in 1969 in Limbo, Panama, from pools containing males or females of unspecified *Lutzomyia* sp. The isolation of the virus from males suggested transovarial and/or sexual transmission within sand flies. Interestingly, CHIV could not adapt to passage through mice, but only through Vero cells. Similarly to PACV, both viruses mentioned above were first described as members of the phlebovirus group; however, their classification was later questioned due to their antigenic differences compared to other phleboviruses [43, 149] and recent molecular and phylogenetic studies reclassified them as belonging to the genus *Pacuvirus* [90, 147].

Rio Preto da Eva virus, the fourth member of the genus *Pacuvirus*, was isolated from unidentified pooled sand flies caught in 1995 in Rio Preto da Eva, Amazonas state, Brazil [145]. To date, information other than the known genome sequence is missing. Similarly, we lack more data on TAPV that was isolated from rodent *Oxymycterus* sp. in Parauapebas municipality, Pará State, Brazil, by the same team [145].

The Santarém virus has been provisionally included in the family *Bunyaviridae* by the ICTV; however, it has not been assigned to any particular genus. Phylogenetic examination showed that this virus is closely related to Caimito virus [150]. Santarém virus was isolated from *Lu. carrerai* and rodents (*Hylaeamys* sp.) [91], but isolation attempts from other sand flies, mosquitoes and biting midgets in 1973 were not successful [151].

Orthobunyavirus (*Bunyavirales*: *Peribunyaviridae*)

The genus *Orthobunyavirus* comprises 103 species [21]. These viruses that infect various vertebrates are transmitted mainly by mosquitoes, but other arthropods, namely biting midges, bed bugs and wingless bat flies, were also identified as vectors. Some species of the genus [e.g. La Crosse virus (*La Crosse orthobunyavirus*), Bunyamwera virus (*Bunyamwera orthobunyavirus*), Cache Valley virus (*Cache Valley orthobunyavirus*), Schmallenberg virus (*Schmallenberg orthobunyavirus*)] cause diseases that are important in human and veterinary medicine [144]. The phylogenetic relationships within the genus *Orthobunyavirus* have recently been changed repeatedly: until 2020, the *Guama virus* serogroup was recognized by the ICTV, comprising four serotypes (Ananindeua, ANUV; Guama, GMAV; Mahogany Hammock, MHV; Moju, MOJUV) [90], but these viruses are now recognized as separate species [20].

To date, the Guamá virus (*Guama orthobunyavirus*, GMAV) is the only member of the genus *Orthobunyavirus* to be isolated from sand flies. The evolution of its classification confuses correct assessment of the published data, as it remains uncertain if the authors of older studies refer to Guamá virus or other members of the 'Guamá orthobunyavirus serogroup'. Guamá virus was first isolated from the blood of sentinel monkey (*Cebus apella*) in 1955, in Oriboca Forest, Pará State in Brazil [152], and it was repeatedly isolated in Brazilian and Caribbean tropical forests [153]. This virus was isolated from a pool of 64 engorged females of *Lu. flaviscutellata* in 1969 in Catu Forest, Pará State, Brazil [107]. It was also isolated from an unspecified *Lutzomyia* sp [154]. However, the same virus or other members of the 'Guamá serogroup' were isolated much more often from various mosquitoes [*Cx. (Melanoconion) taeniopus*, *Cx. (M.) sacchettae*] or rodents (*Hylaeamys* sp.) [155]. According to Woodall [148], GMAV was also isolated repeatedly from other vertebrates (humans, sentinel animals, rodents, marsupials) and mosquitoes

(*Aedes* sp., other mosquitoes) [148]. The Guamá virus is a medically important virus but detailed information about the human GMAV seroprevalence is limited. Travassos da Rosa and Vasconcelos reported 1–2% seropositivity for GMAV and closely related Catú viruses in humans in Brazilian Amazonia, but in some areas (e.g. Breves), almost 50% of people were seropositive [153]. The disease usually lasts for 4–5 days, and patients have acute febrile illness symptoms (fever, dizziness, headache, muscle pain, arthralgia, photophobia) [154, 156]. In experimentally infected laboratory mice, the virus creates lesions in the CNS [148], but in an experimental infection of golden hamsters, the liver was most affected by GMAV [157].

NEGEVIRUS (ICTV UNCLASSIFIED)

Negevirus is a recently described group of probably insect-specific viruses that are not yet recognized by the ICTV. Representatives of this group were isolated from various insects, including sand flies, in geographically distant regions of America, Europe, Africa, Australia and Asia. Their geographical distribution seems to be restricted between 42°N and 42°S latitude, which implies that in addition to the presence of suitable vectors, environmental and climatic conditions could also be significant factors in their occurrence. Virions, most often 45–55 nm in diameter, are of spherical or elliptical shape and contain a non-segmented, positive-sense and polyadenylated ssRNA genome ranging from 9 to 10 kb in size. Three open reading frames (ORFs 1–3), separated by intergenic and untranslated regions, encode viral polymerase protein, glycoprotein and membrane proteins. The position of *Negevirus* in the taxonomical system remains unclear; according to phylogenetic and genomic analyses, it is most related to, but still relatively distant from, plant viruses of families *Kitaviridae* and *Virgaviridae*, which are transmitted by mites, soil nematodes, protozoa, seeds and pollen [158–165]. The connecting link between these groups could be nege-like viruses isolated from aphids [166] or *Fragaria vesca-associated* virus 1 from strawberry plant, which is by its genomic organization and sequence related to negeviruses [167]. Kallies *et al.* [168] demonstrated that the group of *Negevirus* is formed by at least two monophyletic groups at the genus level: *Nelorpivirus* and *Sandewavirus*.

The life cycle of *Negevirus* in nature remains unknown but seems to be linked to insects. Until recently, negeviruses have been found to mainly be associated with mosquitoes (species of genera *Culex*, *Aedes*, *Anopheles*, *Armigeres*, *Psorophora* spp., *Uranotaenia*, *Deinocerites*, *Wyeomyia* and *Trichoproson*) [159–161, 168–172] and sand flies (*Lutzomyia* spp.) [159, 160]. However, these observations can be biased by sampling during arbovirus surveillance studies, resulting in an underestimation of the actual host range [159]. New studies revealed negeviruses in *Glossina morsitans morsitans* (GmmNegeV) [173] and in a pool of biting midges *Culicoides* spp. (Turkana_9) [174]. Furthermore, other negeviruses were detected in the dungfly (*Scathophaga furcata*) by next-generation sequencing, and in various members of Insecta, Arachnida, Malacostraca, Maxillopoda, Chilopoda, Nematoda and Cnidaria by searching various bioinformatics databases [175, 176].

To date, there have only been two detections of a *Negevirus* from sand flies. The first negevirus isolated from sand flies is called *Loreto* virus (genus *Nelorpivirus*, strain 2617/77) obtained from a pool of *Lutzomyia* spp. in Peru in 1977 [160]. Nevertheless, *Loreto* virus is not sand fly-specific; strains 3940–83 and PeAR 2612/77 were also isolated from pools of *An. albimanus* in 1983 and *Culex* sp., respectively, both isolations performed in Peru in 1977 [160]. The second negevirus from sand flies is called Piura virus (strain CoR10) and its RNA was obtained from *Lu. evansi* sampled in 2013 in Florida, USA. Similarly like the Loreto virus, the Piura virus was also detected in other hosts, namely mosquitoes [159].

Despite this question still being unresolved, we may expect a certain degree of species specificity within this group as a prototype of Negev virus (EO239), isolated in Israel 1983 from *An. coustani* pool, successfully infected and replicated in cells of *Ae. albopictus* (C6/36 and C7/10), *An. albimanus*, *Cx. tarsalis* and *Ph. papatasi*, whereas in *An. gambiae* and *Drosophila melanogaster* cells, the virus titres stagnated or even declined during the first 72 h p.i. Furthermore, the impact of Negev virus infection on cells differs; the CPE was only visible in *Aedes* and *Culex* cell lines but not in *Ph. papatasi* or *An. albimanus* [160]. Another virus belonging to this group, the Uxmal virus, was isolated from *Ae. taeniorhynchus* in Mexico and successfully replicated in C6/36 (with visible CPE) and *Cx. tarsalis* (without CPE) cells, but not in *An. gambiae* (Sua 4.0) and lepidopteran cells (Sf9, High Five) [177], further fostering the possibility of certain host specificity.

Isolation of a negevirus, namely Okushiri virus, from *Aedes* larvae indicates that either oral infection of larvae through plant detritus and/or microbes or transovarial transmission exists [178]. The second possibility is also supported by the detection of another negevirus (Castlerea virus) in *Culex* spp. males [162]. This hypothesis is also supported by recent findings on the Massilia virus [179], which suggest that some viruses are able to infect sand flies and mosquitoes through their natural sugar sources, such as plant sap or honeydew. However, the detection of negevirus in the tsetse fly [173] does not support this hypothesis, as tsetse flies are exclusively haematophagous. Alternative hypotheses about the natural circulation of negeviruses involve ectoparasitic mites of mosquitoes and sand flies [159, 178, 180, 181].

Our knowledge of negeviruses is so far limited and many aspects remain unclear. Future research shall address several important questions. Do mosquitoes and sand flies serve as vectors or are they just dead-end hosts of plant viruses? Or vice versa – are negeviruses insect symbionts or parasites with plants serving as reservoirs of infection? Could the presence of these viruses affect the vector competence for medically important arboviruses, as known for some other insect-specific viruses (reviewed in [182])?

And what may be the effect of coinfection and superinfection phenomenon? The last question was addressed by Patterson *et al.* [183], who studied the superinfection of *Ae. albopictus* (C7/10) cell lines infected by various alphaviruses (Venezuelan equine encephalitis virus, Chikungunya virus, Semliki Forest virus, O'nyong-nyong virus, Mayaro virus) and subsequently by one of the three strains of negevirus (NEGEV-M30957, PIUV-EVG 7–47, PIUV-CO R 10). The alphavirus titres were reduced at 48 hours p.i. for all combinations of virus superinfections. However, the differences in the alphavirus titres reductions were observed for each combination of alphavirus–negevirus. The smallest effect on titre reduction was obtained for the combination of Chikungunya virus (CHIKV) with NEGEV-M30957. On the other hand, when the authors of the same study created a fused recombinant virus, NEGEV-M30957, expressing GFP and a partial sequence for an anti-CHIKV antibody (NEGEV scFv-CHK265) and used it for superinfection of CHIKV infected cells, the CHIKV titres decreased rapidly [183]. The results of this study suggest that negeviruses may potentially be used for pathogen control, either by natural inhibition of other viruses or, in their modified form, to control a specific pathogen in a vector.

VECTORIAL ROLE OF SAND FLIES

Despite the fact that the role of phlebotomine sand flies in the transmission of various viruses has been known for decades, our understanding of their actual involvement in particular transmission cycles, the degree of vector specificity and other details remain fragmentary and rather poorly understood when compared with vast knowledge of their role in transmission of parasitic and disease-causing *Leishmania*. The reasons for this rather unsatisfactory state of knowledge are manifold. As outlined earlier, the incrimination of sand flies as vectors of viruses is based on four rigorous criteria [14] that include not only evidence from the field, but also experimental data for which the availability of laboratory colonies is necessary. In this respect, only a handful of sand fly species were conclusively proven to be vectors of viruses while others shall be considered as suspected or potential vectors.

The fact that only a fraction of sand fly species are bred under laboratory conditions and available for experimental infections poses serious constraints for the assessment of their role in the transmission of viruses. This is especially true for New World vectors, where only five species of the genus *Lutzomyia* are currently reared in captivity [184]. Of these, by far the most frequently bred species is *Lu. longipalpis*, which itself represents a taxonomically challenging complex of cryptic species, with only some of these being formally described, and which may differ in their capacity to transmit pathogens, including viruses [185]. Therefore, experimental data based on these colonies shall be treated with caution and aligned to the actual strains or cryptic species from which they were yielded. At some instances, to overcome the unavailability of a laboratory colony of a potential vector species for experimental assays, freshly 'colonized' sand flies collected from a monospecific locality were used in the past studies, as done with the *Lu. trapidoi* to demonstrate their ability to transmit VSIV [48]. However, such experimental design may be affected by the undetected natural infection of the sand flies used and shall therefore be avoided. For similar reasons, freshly established colonies must be scrutinized for the presence of viral infection due to potential transovarial transmission, which was proposed as a bias affecting the experimental assessment of Chandipura virus transmission by *Ph. argentipes* [76]. In general, a much wider selection of well-characterized laboratory colonies of various sand fly species bred under standard conditions and available for experimental assays appears to be a key factor that would greatly improve our understanding transmission of sand fly-borne viruses.

Data inferred from the field-collected sand flies are of equally crucial importance to understand the vectorial role of different sand fly species; however, in many studies, they are of limited value due to the collection methodology; as summarized in Table 2, some viruses were isolated more often from pools of either unidentified sand flies or from pools comprising several species co-occurring at the trapping site. In addition to incidental findings of rare viruses such as the Iriri virus that was only once isolated from an unidentified pool of *Lutzomyia* spp. [91] and never detected again, this also applies to more thoroughly known viruses of medical relevance. As summarized above, Chandipura virus, a causative agent of acute encephalitis syndrome in India, was repeatedly isolated solely from unidentified sand fly pools. Saboya virus was detected in Senegal from a polyspecific pool of sand flies at a study site where the sand fly fauna is composed of only one species of the genus *Phlebotomus* but no less than nine species of the genus *Sergentomyia* that belong to at least three subgenera; while *Ph. duboscqi* occurs in low numbers throughout the season, several *Sergentomyia* species (*Se. antennata*, *Se. buxtoni*, *Se. clydei*, *Se. dubia*, *Se. magna* and *Se. schwetzi*, which is the dominant species) are each abundant enough to be potentially incriminated in the transmission of SABV [64]. These examples illustrate that even though such an approach attracts methodological, logistical and also budgetary difficulties, the aim of field studies is to provide analyses of well-defined and ideally monospecific sand fly collections in order to provide accurate insights into the vectorial role of different sand fly species in the transmission of the studies sand fly-borne viruses.

There are hints stemming from both field and experimental studies suggesting that some viruses are indeed transmitted by their specific sand fly vectors. Santarém virus was isolated from *Lu. carrerai* [91], but not from other sand flies, mosquitoes and biting midgets from the same area [151], Pacui virus was isolated from pools of *Lu. flaviscutellata* but not from pools of local mosquitoes or from *Lu. infraspinosa*, the second most abundant local sand fly species [107]. Carajas virus, which was originally isolated from a pool of unidentified *Lutzomyia* spp., could not be experimentally transmitted by *Lu. longipalpis* from a laboratory colony, suggesting that other sand fly species serve as its natural vectors. This consideration was further supported by the fact that Maraba

Table 2. Viruses detected in phlebotomine sand flies

Virus species (genus, order: family)	Virus name	First isolated from sand fly species	First place of virus isolation (sand fly sampling year)	Reference
Vesiculovirus (Mononegavirales: Rhabdoviridae)				
Vesiculovirus alagoas	Vesicular stomatitis Alagoas virus (VSAV)	*Lutzomyia* spp.	Colombia (1986)	[26]
Vesiculovirus indiana	Vesicular stomatitis Indiana virus (VSIV)	*Lutzomyia* spp.	Almirante, Panama, (1959–1962)	[42]
Vesiculovirus newjersey	Vesicular stomatitis New Jersey virus (VSNJV)	*Lu. shannoni*	Ossabaw Island, Georgia, USA (1988)	[40]
Vesiculovirus chandipura	Chandipura virus (CHPV)	*Phlebotomus* spp.	Aurangabad, Maharashtra State, India (1969)	[72]
Vesiculovirus isfahan	Isfahan virus (ISFV)	*Ph. papatasi*	Isfahan province, Iran (1975)	[84]
Vesiculovirus carajas	Carajas virus (CJSV)	*Lutzomyia* spp.	Maraba, Para State, Brazil (1983)	[86]
Vesiculovirus maraba	Maraba virus (MARV)	*Lutzomyia* spp.	Maraba, Para State, Brazil (1983)	[86]
Vesiculovirus bogdanovac	Yug Bogdanovac virus (YBV)	*Ph. perfiliewi*	Dobrić, Mačva District, Serbia (1976–1982)	[89]
Vesiculovirus morreton	Morreton virus (MORV)	*Lutzomyia* spp.	Durania, Colombia (1986)	[26]
Vesiculovirus perinet	Perinet virus (PERV)	*Ph. berentiensis*[*]	Périnet, Madagascar (1978)	[191]
Vesiculovirus radi	Radi virus (RADV)	*Ph. perfiliewi*	Radi, Tuscany, Italy (1982)	[192]
Curiovirus (Mononegavirales: Rhabdoviridae)				
Curiovirus iriri	Iriri virus	*Lutzomyia* spp.	Altamira, Para, Brazil (1982)	[91]
Sripuvirus (Mononegavirales: Rhabdoviridae)				
Sripurvirus naikha	Niakha virus (NIAV)	*Ph. duboscqi* and *Sergentomyia* sp.	Niakha, Senegal (1992)	[64]
Sripurvirus charleville	Charleville virus (CHVV)	*Phlebotomus* sp.	Charleville, Queensland, Australia (1969)	[99]
Sripurvirus sripur	Sripur virus (SRIV)	*Sergentomyia* sp.	West Bengal, India (1973)	[97]
Arurhavirus (Mononegavirales: Rhabdoviridae)				
Arurhavirus inhangapi	Inhangapi virus (INHV)	*Lu. flaviscutellata*	Catu Forest, Pará State, Brazil (1969)	[107]
Arurhavirus santabarbara	Santa Barbara virus (SABV)	Uncharacterized Psychodidae	Pará state, Brazil (2010)	NCBI GenBank Ref. Sequence: NC_028234.1
Flavivirus (Amarillovirales: Flaviviridae)†				
Saboya virus	Saboya virus (SABV)	Uncharacterized Phlebotominae	Ferlo, Senegal (1992)	[64]
Ecuador Paraiso Escondido virus	Ecuador Paraiso Escondido virus (EPEV)	*Lu.* (*Psathyromyia*) *abonnenci*	Pichincha province, Ecuador (2011)	[114]
Orbivirus (Reovirales: Sedoreoviridae)				
Changuinola virus	Changuinola virus (CGLV)	Uncharacterized Phlebotominae	Almirante, Panama (1959–1962)	[42, 136]
Pacuvirus (Bunyavirales: Peribunyaviridae)				
Pacui pacuvirus	Pacui virus (PACV)	*Lu. flaviscutellata*	Catu and Utinga Forest, Pará State, Brazil (1968)	[107]
Caimito pacuvirus	Caimito virus (CAIV)	*Lu. ylephilator*	El Aguacate, Panama (1971)	[43]
Chilibre pacuvirus	Chilibre virus (CHIV)	*Lutzomyia* sp.	Limbo, Panama (1969)	[43]
Rio Preto da Eva pacuvirus	Rio Preto da Eva virus (RPEV)	Uncharacterized Phlebotominae	Rio Preto da Eva, Amazonas state, Brazil (1955)	[41]
Related but unclassified	Santarém virus (STMV)	*Lu. carrerai*	Brazil (NA)	[91]
Orthobunyavirus (Bunyavirales: Peribunyaviridae)				
Guama orthobunyavirus	Guamá virus (GMAV)	*Lu. flaviscutellata*	Catu Forest, Pará State, Brazil (1969)	[107]

[*]In the original publication, the authors referred to this species as *Sergentomyia berentiensis* [191].
†Only dual-host or putative dual-host flaviviruses are mentioned.

virus isolated from another unspecified pool of *Lutzomyia* spp. females caught in the same area was replicated successfully in *Lu. longipalpis* after intrathoracic infection and was transmitted to progeny [86].

However, other viruses may be far less specific. Changuinola virus was repeatedly isolated from pools containing either unidentified *Lutzomyia* sp. specimens or monospecific pools of *Lu. trapidoi* and *Lu. ylephilator* females, respectively, suggesting that it may be transmitted by both species and potentially also by other sand flies not identified in the original mixed pool. Charleville virus was isolated from unidentified sand flies, but it was also detected in *Forcipomyia* sp. biting midges in Queensland, Australia [99]. Some other examples include Guamá virus isolated from *Lutzomyia* spp. sand flies but also from several *Culex* mosquito species [155] and Loreto virus, which, besides being isolated from yet again unidentified *Lutzomyia* spp., is also known from pools of *An. albimanus* in 1983 and *Culex* sp., respectively; both isolations were performed in Peru in 1977 [160]. Again, further field research complemented by experimental efforts using laboratory colonies would elucidate the degree of vector specificity of these viruses.

The aforementioned gaps in knowledge regarding the role of respective sand fly species in the transmission of different viruses are further emphasized by the changing distribution of the potential vectors due to climatic and environmental changes linked to the human-caused alteration of the global climate. Like other arthropod vectors, sand flies are expected to react to ongoing processes triggered directly or indirectly by climate change and their changing distribution is suggested in different regions, including North America [186], Europe [187] and North Africa [188]. Fragmentary knowledge of temporal and spatial distribution due to the lack of consistent and detailed mapping of various sand fly species has not so far provided large sets of robust field-derived data, but the assumptions concerning changing sand fly geographical ranges are supported by various modelling approaches, mainly ecological niche modelling (ENM). For example, *Lutzomyia flaviscutellata*, a species incriminated in the transmission cycle of several viruses, is projected to expand into new regions as well as higher altitudes in several countries of the Amazonian region, exposing large human populations to the risk of sand fly-borne pathogens transmission [189]. A general expectation of sand flies emerging into new regions, however, may oversimplify the more complex processes; ENM also suggests a potential reduction in the spatial distribution of two species of the genus *Lutzomyia* in Colombia [190]. These examples emphasize the need for detailed surveillance of sand flies in endemic and adjacent countries and a sustained focus on studying their involvement in the transmission cycles of sand fly-borne pathogens, including viruses, in the future.

CONCLUSION

(1) In the literature search, 26 viruses other than members of the genus *Phlebovirus* were isolated or detected in phlebotomine sand flies.
(2) These 26 viruses belong to 8 genera.
(3) Most of these have been detected quite occasionally, and more detailed information about their biology or pathology with respect to vertebrate hosts is therefore lacking and research on these viruses is generally neglected.
(4) Some sand fly-borne viruses are nevertheless of high medical and veterinary importance (Vesicular stomatitis virus complex, Changuinola virus and Guamá virus).
(5) The vectorial role of phlebotomine sand flies for different viruses varies; some viruses were isolated from several sand fly species or even from other bloodsucking arthropods (Charleville virus, Changuinola virus, Chandipura virus, Saboya virus, etc.), but on the other hand, several other viruses seem to be more vector-specific (Santarem virus, Pacui virus).
(6) Some of the newly discovered insect-specific viruses have also been detected in phlebotomine sand flies. Research on their potential for changing vector competence during coinfection or superinfection with vertebrate–pathogenic viruses can bring promising results, as formerly shown for mosquito-specific viruses.

Funding information
This study was supported by a research project funded by the project National Institute of Virology and Bacteriology (programme EXCELES, ID project no. LX22NPO5103), funded by the European Union – Next Generation EU.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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