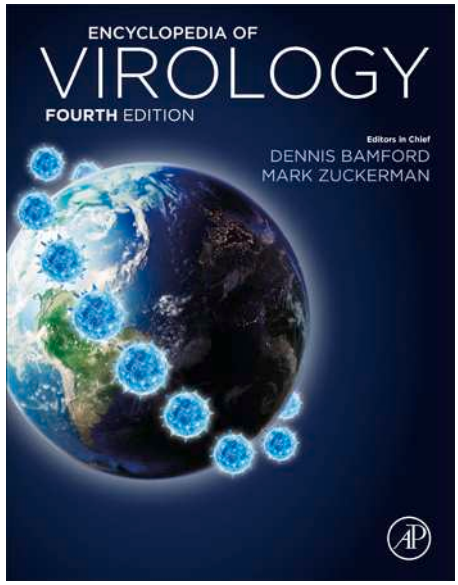

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Pecluviruses (*Virgaviridae*)

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Nomenclature

aa Amino acid(s)
AGO Argonaute 1
CITE Cap-independent translation enhancer
Co-Pro Protease-cofactor
CP Coat protein or capsid protein
CRP Cysteine-rich protein
ELISA Enzyme-linked immunosorbent assay
ER Endoplasmic reticulum
HC-Pro Helper component-proteinase
IRES Internal ribosome entry site
kb Kilobase
kDa Kilodalton
LAMP Loop-mediated isothermal amplification
mAbs monoclonal antibodies
MP Movement protein
NCR Noncoding regions
nt Nucleotide(s)
OAS Origin of assembly
ORF Open reading frame
pAbs Polyclonal antibodies
PCR Polymerase chain reaction
RdRp RNA-dependent RNA polymerase
RISC RNA-induced silencing complex
RT-qPCR Reverse transcription quantitative PCR
satRNA satellite RNA
UTR Untranslated region
VIGS Virus-induced gene silencing
VLPs Virus-like particles
VPg Viral protein genome-linked
VRC Virus replication complex
vRNA virion RNA

Glossary

Forma specialis An informal rank in a classification, allowed by the International Code of Nomenclature for Algae, Fungi, and Plants, that is applied to a parasite (most frequently a fungus), which is characterized from a physiological standpoint (e.g., by the ability to infect a specific host), but scarcely or not at all from a morphological standpoint.
Fortuitous hosts of obligate biotrophic Plasmodiophorida Host on which some primary infections evolve into resting spores without going through the zoosporangial stage.
Hetero-encapsidation Partial or full coating of the genome of one virus with the coat protein of a differing virus. Also termed trans-capsidation or heterologous encapsidation.
Leaky scanning Mechanism by which the ribosomes fail to initiate translation at the first AUG start codon and scan downstream for the next AUG codon.
Obligate endoparasite A parasitic organism that cannot complete its life cycle without exploiting a suitable living host, and that lives within that host.
Post-transcriptional gene silencing Mechanism for sequence-specific RNA degradation in plants.
Triple gene block A specialized evolutionarily conserved gene module involved in the cell-to-cell and long-distance movement of plant viruses.

History

Peanut (groundnut, *Arachis hypogaea*) clump disease, characterized by severe stunting and clumping, was first described from India in 1927 and subsequently from West Africa in 1931. The causal agent in West Africa as well as in India was identified as a virus, *Peanut clump virus* (PCV) and *Indian peanut clump virus* (IPCV), respectively. Annual losses to the peanut crop due to PCV and IPCV were estimated to exceed US$38 million in the late 1990s. Pecluviruses also cause diseases on several dicotyledonous and monocotyledonous crops.

Taxonomy and Classification

The genus *Pecluvirus* has only two species, isolates from West Africa were grouped under the species *Peanut clump virus* (Peanut clump virus; PCV) and those from the Indian subcontinent were grouped under the species *Indian peanut clump virus* (Indian peanut clump

virus; IPCV). The two virus species were initially assigned to the genus *Furovirus* mainly based on virion morphology and vector transmission by *Polymyxa graminis* (*Rhizaria*, *Phytomyxea*, *Plasmodiophorida*). Subsequently, based on molecular features, the ICTV in 1997 assigned PCV and IPCV to the newly established genus *Pecluvirus* (siglem from Peanut clump virus). They differ in their geographical distribution, host range, antigenic properties and genomic sequences. Later, the genera *Tobamovirus*, *Tobravirus*, *Hordeivirus*, *Furovirus*, *Pecluvirus*, *Pomovirus*, and *Goravirus* were assigned to the newly established family *Virgaviridae* (from the Latin Virga, meaning rod).

Geographic and Seasonal Distribution

PCV occurs in Burkina Faso, Benin, Chad, Congo, Côte d'Ivoire (Ivory Coast), Niger, Mali, Gabon, Senegal, and Sudan, and IPCV in the Indian states of Punjab, Andhra Pradesh, Telangana, Tamil Nadu, Rajasthan, Haryana, and Gujarat, and in Pakistan (Sind and Punjab provinces). IPCV and PCV infections are noticed mainly in the peanut crops cultivated during the rainy season (July–November in India and April–October in West Africa). Nevertheless, post-rainy season crops can also be infected.

Host Range and Symptoms

PCV infects peanut, cowpea, sugarcane, maize, sorghum, pearl millet, and finger millet, whereas IPCV infects peanut, cowpea (*Vigna unguiculata*), pigeonpea (*Cajanus cajan*), chili (*Capsicum annuum*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), and finger millet (*Eleusine coracana*). *Cynodon dactylon*, *Cyperus rotundus*, *Oldenlandia aspera*, and *Vigna subterranea* (bambara groundnut) that can play a significant role in both virus survival and dissemination.

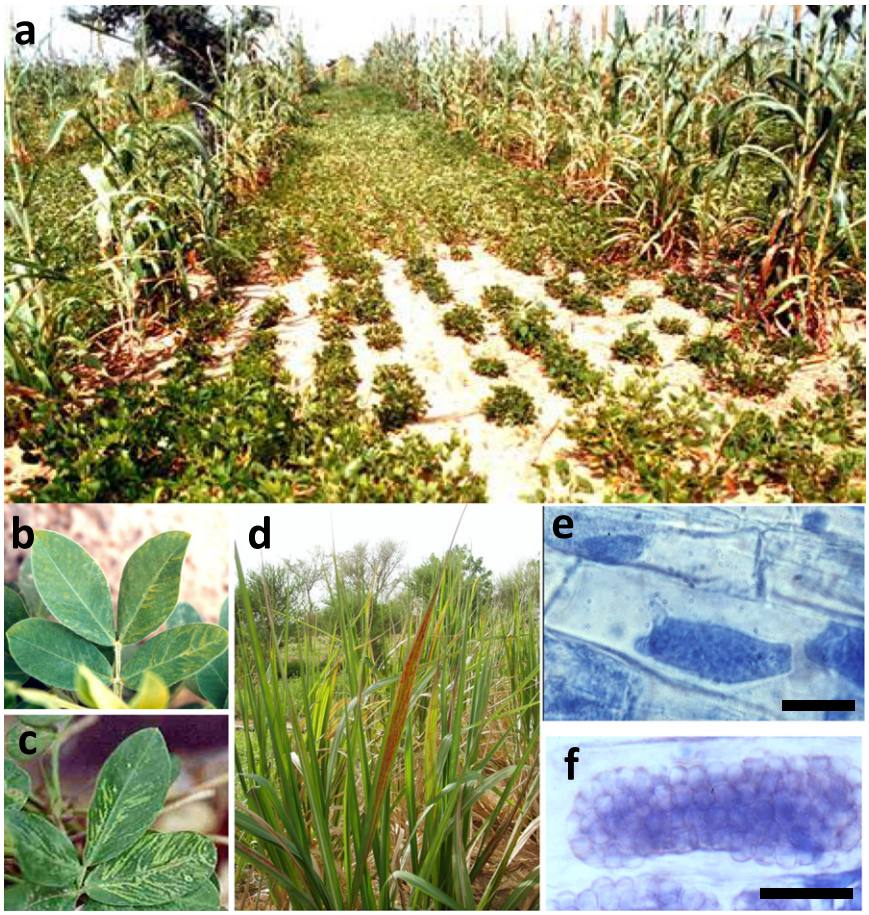

Fig. 1 Symptoms induced by Peanut clump virus (PCV), in West Africa. (a) field-infected peanut plants occurring in patches of various sizes, intercropped with pearl millet in Maradi, Niger Republique, (b) peanut leaflets showing initial symptoms of chlorosis and mild mosaic, (c) chlorosis along the veins resembling oak leaf, (d) PCV-infected sugarcane plants exhibiting red leaf mottle in Burkina Faso, (e) IPCV and PCV vector, *Polymyxa graminis*, in transversal section of sorghum roots, stained with lactophenol blue showing zoosporangia, (f) sporosori, bars represent 25 μm. Photographs courtesy of P. Delfosse.

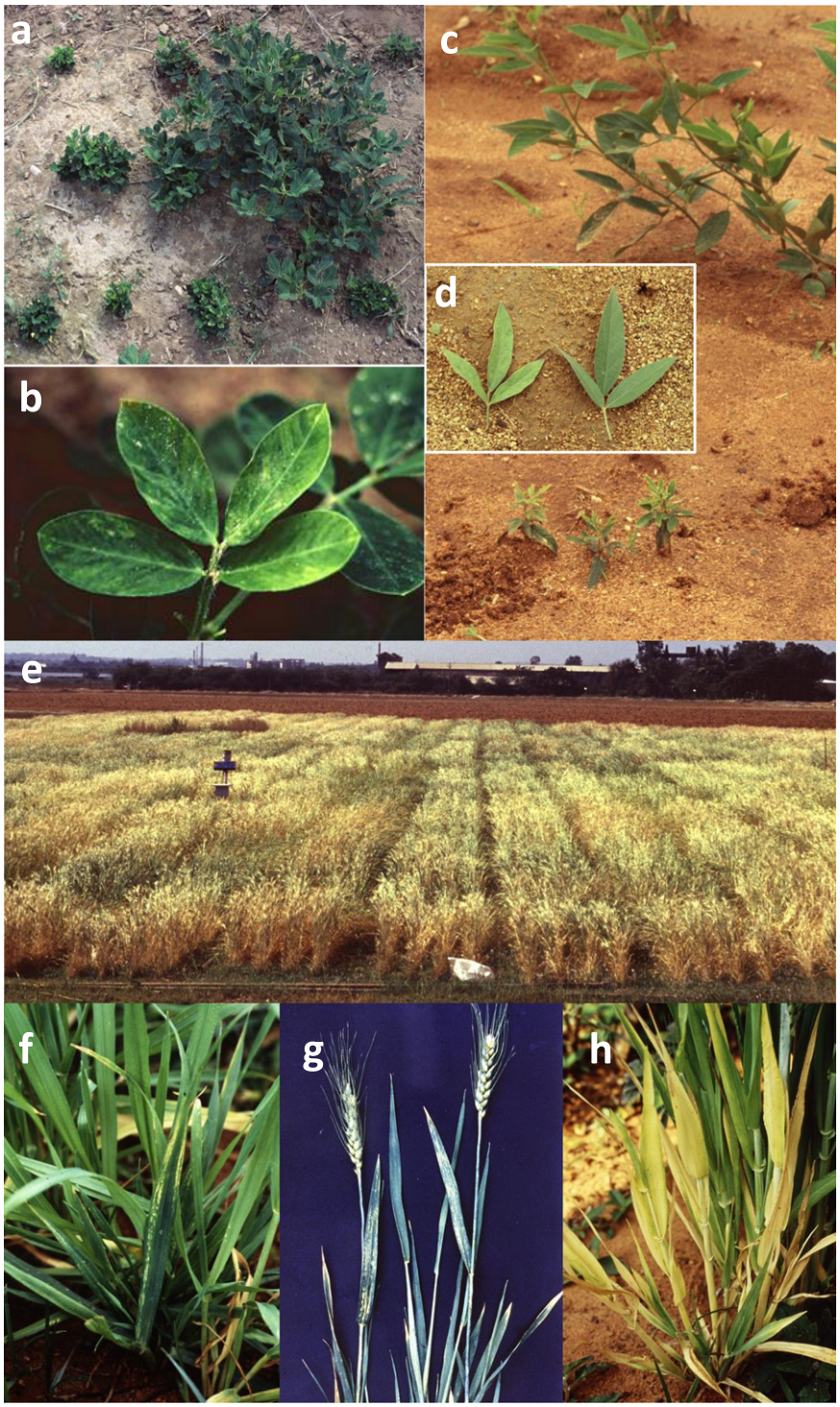

Fig. 2 Symptoms induced by Indian peanut clump virus (IPCV) on various hosts. (a) on peanut plants showing severe stunting, occurring in patches in the field, and an apparently uninfected plant, (b) young peanut leaflets with initial symptoms of chlorosis and mosaic symptoms, (c) early infected pigeonpea plants showing severe stunting with an apparently uninfected plant, (d) inset showing chlorotic symptoms on young pigeonpea leaflets (left) and by its side leaflets from healthy plant, (e) infected wheat plants occurring in patches, (f) early infected wheat plants showing dark green leaves and chlorotic streaks, (g) late infected wheat plants showing panicle formation. Seed derived from them can transmit IPCV up to 1%, (h) early infected barley plants showing stunting and severe yellowing. Photographs courtesy of P. Delfosse.

Diseased peanut plants are conspicuous under field conditions because of their dark green appearance, stunting, and occurrence in patches (Fig. 1(a) and Fig. 2(a)). The disease incidence is high in fields where peanut crops are grown in rotation with wheat, barley, and pearl millet. Early-infected peanut plants often did not yield pods and late-infected crops showed decreased crop yields up to 60%.

In peanut, the symptoms incited by PCV are essentially similar to those induced by IPCV. In West Africa, peanut clump disease symptoms are often confused with green rosette because of the presence of stunted dark green plants occurring in patches in the fields. The peanut clump disease occurs in the same areas year after year with marginal increase in the periphery of the patches in the successive peanut crops. Initial symptoms on young leaflets of peanut are mottling, mosaic and chlorotic veins or the 'oak leaf symptom' (Fig. 1(b) and (c), Fig. 2(b)). When such leaflets mature, they turn dark green with or without faint mottling. Late infected plants may not show conspicuous symptoms and clumping is restricted to a few branches in some plants. PCV causes the red leaf mottle disease in sugarcane (*Saccharum officinarum*) (Fig. 1(d)).

Pigeonpea plants infected with Hyderabad isolate of IPCV (IPCV-H) showing stunting are presented in Fig. 2(c). Fig. 2(d) shows chlorosis on infected pigeonpea leaflets, left, and uninfected leaflets, right. Wheat plants, up to three weeks old, infected by IPCV-H show rosette symptoms similar to those caused by the *Soil-borne wheat mosaic virus* (SBWMV) and grain yield losses up to 58% are recorded. Wheat cultivar RR-21 infected with IPCV-H showing stunting is presented in Fig. 2(e). Early infected wheat plants show chlorotic streaks (Fig. 2(f)) and will produce panicles (Fig. 2(g)). Seed from these plants can transmit IPCV up to 1%. Barley plants infected with IPCV-H are stunted and bushy with chlorotic or necrotic leaves (Fig. 2(h)) and the majority of these plants die. The plants that reached maturity produced poorly developed spikes. IPCV-H also infected pearl millet, finger millet, foxtail or Italian millet, and sorghum plants. In maize IPCV-H caused aerial biomass losses up to 33% and grain loss up to 36%.

Various isolates of PCV and IPCV are readily detected in the cells of roots, stems, and leaves of systemically infected hosts. In wheat cells, PCV particles are found in the cytoplasm, near the nucleus or along the plasmalemma, and arranged in angled-layer aggregates.

In experimental host range studies by sap inoculation, PCV and IPCV infected a wide range of both dicotyledonous and monocotyledonous plants. For IPCV, *Nicotiana clevelandii* x *N. glutinosa* hybrid and *Phaseolus vulgaris* (cv. Top crop) are suitable for virus propagation and as assay/diagnostic hosts, respectively. *Chenopodium amaranticolor* and *C. quinoa* are local lesions hosts. IPCV isolates collected from clump diseased peanut crops from different locations in India differ slightly in their host ranges. *Canavalia ensiformis* and *N. clevelandii* x *N. glutinosa* hybrid are found to be useful for differentiating the isolates of IPCV. In the case of PCV, *C. amaranticolor*, *N. benthamiana*, *N. glutinosa*, *P. vulgaris*, and *Triticum aestivum* are of diagnostic value. *N. benthamiana* and *P. vulgaris* are suitable for virus propagation. The symptoms induced in *C. amaranticolor* by various PCV isolates collected from Burkina Faso, Senegal, and Niger are shown to differ markedly.

Transmission

Seed and Sap Transmission

Pecluviruses can be transmitted by sap inoculation and through peanut seed. The transmission rate through the peanut seed depends on the cultivar, the mode of infection (up to 24% for plants infected through soil and up to 50% for plants infected through seed) and the age at which the plants are infected. IPCV is transmissible up to rates of $<2\%$ through the seeds of pearl millet, finger millet, foxtail millet, wheat, and maize. Pearl millet CV GGP-16 did not transmit IPCV through seed.

Soil-Borne Vector Transmission

Both PCV and IPCV are naturally vectored by soil-borne protists, *P. graminis* (Fig. 1(e) and (f)). Two formae speciales of this species are associated with the transmission of PCV and IPCV: *P. graminis*. f. sp. *subtropicalis* and *P. graminis* f. sp. *tropicalis*. These obligate root endoparasites complete their entire life cycle, including sporangial and sporogenic (resting spores) phases (Fig. 1(e) and (f); Fig. 3 right panel), only in monocotyledonous hosts in the family *Poaceae* (*P. graminis tropicalis* on sorghum, pearl millet, and maize but rarely on wheat and barley and *P. graminis subtropicalis* on sorghum, pearl millet, wheat, and barley). Nonetheless, they can infect and transmit viruses to dicotyledonous hosts, including peanut, which are considered to be fortuitous hosts of the vector. The presence of *P. graminis* in infested soils can be identified by growing bait plants such as pearl millet, sorghum, or wheat on infested soils or by PCR. *In vitro* transmission studies under controlled conditions is complicated because of the obligate parasitic nature of *P. graminis* and consequent difficulties in maintaining the cultures of both the virus and the vector in the same hosts. Nevertheless, the acquisition and the transmission of PCV by the vector is demonstrated using certain hosts such as sugarcane (CV CP-89–2377) and *Sorghum arundinaceum* supporting abundant zoospore production and virus infection. Presence of *P. graminis* in roots can also be quantified by reverse transcription quantitative PCR (RT-qPCR). The P39 viral protein, encoded by RNA2 and expressed by leaky scanning of P23, is involved in the transmission by *P. graminis* based on its similarity with P75 protein encoded by RNA2 of *Beet necrotic yellow vein virus* (BNYVV).

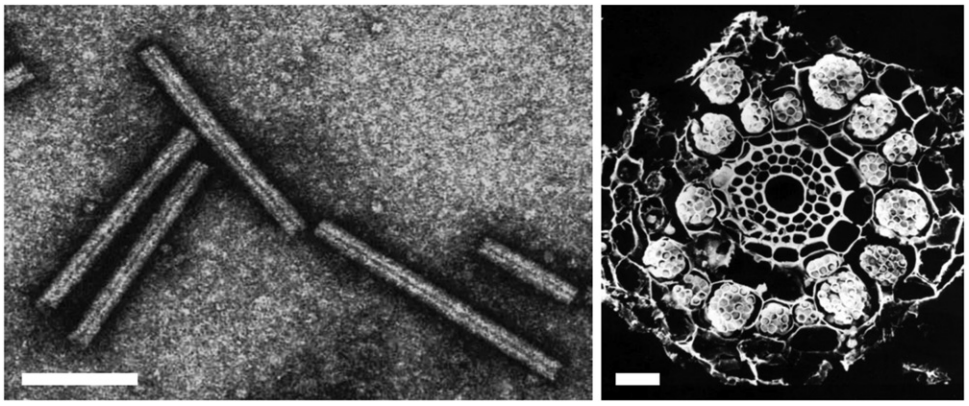

Fig. 3 Electron micrographs of the Indian Peanut clump virus (IPCV; *Pecluvirus*, *Virgaviridae*) and its vector *Polymyxa graminis*. (left) Virus particles with two predominant lengths of 249 and 184 nm are decorated with homologous antiserum for the Hyderabad isolate, bar represents 100 nm. (right) Transversal section of a sorghum root heavily infested by *P. graminis* showing sporosori, bar represents 25 μm. Photographs courtesy of P. Delfosse and Larry Littlefield.

Virion Morphology

The virions of IPCV (Fig. 3 left panel) and PCV are non-enveloped rigid straight rod shaped, 24 nm (IPCV) and 21 nm (PCV) in diameter with two predominant lengths of c. 250 and c. 180 nm. They have helical symmetry with a pitch of 2.6 nm and contain a single coat protein (CP) of 23 kDa.

Physical Properties

Virions sediment as two major components with S_{20w} of 183S and 224S. Buoyant density in CsCl is 1.32 g cm^{-3} and isoelectric point is pH 6.45. Thermal inactivation of virus infectivity occurs at 64°C. Virions are stable in frozen leaves.

Molecular Properties

Genome Structure and Expression Strategies

The genome of pecluviruses is bipartite, with two positive-sense, linear single-stranded RNAs (ssRNAs) (4% in weight). RNA1 is c. 5900 nt long and RNA2 is c. 4500 nt long. The PCV RNAs show little sequence identity with those of IPCV RNAs except for the 3′ terminal 273 nt, which are conserved among the two RNAs. Both the RNAs have methylated cap structure (to be confirmed) at their 5′ ends. The 3′ ends of the RNAs have tRNA-like structure and are not polyadenylated. Both RNAs are flanked by untranslated regions (5′- or 3′ UTR) or noncoding regions (5′- or 3′ NCR). The genome organization and expression of the PCV is shown in the Fig. 4.

Coding Sequences

RNA1 contains two open reading frames (ORFs) that encode two proteins involved in viral RNA replication (P131 and P191, Fig. 4; Table 1). The proximal ORF encodes a 131 kDa polypeptide and P191 is a C-terminally extended form of P131 produced by translational readthrough of the UGA termination codon. The P15 ORF is downstream of the P191 ORF and separated from it by a noncoding region of about 60 nt. The polypeptides of 131 and 191 kDa contain NTP-binding methyl transferase, helicase and RNA-dependent RNA polymerase (RdRp) domains and are involved in the putative replication complex (Table 1). The 15 kDa polypeptide, a cysteine-rich protein (CRP) is translated from a sub-genomic RNA (Fig. 4). It is a suppressor of post-transcriptional gene silencing (PTGS). The P15 possesses four C-terminal proximal heptad repeats that can generate a coiled-coil interaction and is targeted to peroxisomes via a C-terminal Ser-Lys-Leu (SKL) motif. Such a motif is conserved among pecluviruses from both Africa and India. It has been demonstrated that a coiled-coil motif is necessary for the anti-PTGS activity of P15, but the peroxisomal localization signal is not, although it is required for efficient intercellular movement of the virus. P15 resembles CRPs of *Barley stripe mosaic virus* (BSMV), *Poa semilatent virus* (PSLV), and SBWMV. These proteins have been suggested to act as regulatory factor during virus replication as well as for long-distance movement and contribute to the virulence factor. RNA1 can replicate in the absence of RNA2 in protoplasts of tobacco BY-2 cells. However, both RNAs are required for infection and systemic invasion of plants. Experiments using enhanced 5′ green fluorescent protein and 5′-bromouridine 5′-triphosphate labels have suggested that PCV replication complexes co-localize with endoplasmic reticulum (ER) bodies accumulating around

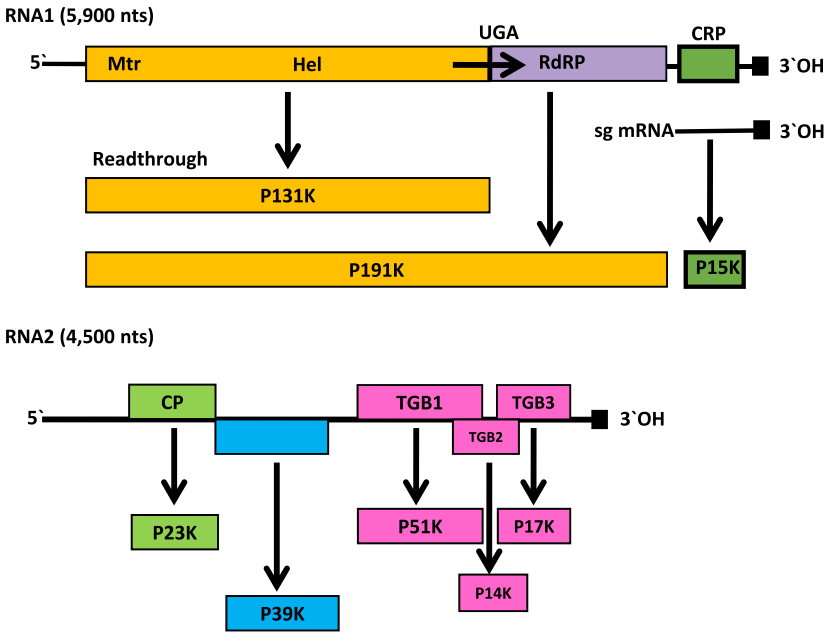

Fig. 4 Genome organization of PCV (genus *Pecluvirus*, family *Virgaviridae*). The tRNA structure motifs at the 3′ termini of the RNAs are represented by a dark square. ORFs are indicated by rectangles and a suppressible termination codon (UGA) by an arrow. Mtr, methyl transferase; Hel, helicase; RdRp, RNA-dependent RNA polymerase; CRP, cysteine-rich protein; CP, coat protein; TGB, triple gene block. Modified from Adams, M.J., Adkins, S., Bragard, C., *et al.*, 2017. ICTV virus taxonomy profile: Virgaviridae. Journal of General Virology 98, 1999–2000.

Table 1 Pecluvirus open reading frames (ORFs), polypeptides and their functions

Genomic RNA	*ORF*	*M_r of polypeptide (kDa)*	*Function*
RNA1	P131	131	Methyltransferase, helicase, replicase
	P191	191	
	P15	15	Suppressor of PTGS
RNA2	P23	23	Coat Protein
	P39	39	Putative vector transmission factor
	P51	51	Virus movement (triple gene block)
	P14	14	
	P17	17	

the nucleus during infection. P15 does not act directly at sites of viral replication but intervenes indirectly to control viral accumulation levels.

RNA2 contains five ORFs (Fig. 4). The 5′ proximal ORF (23 kDa) encodes the CP, and the adjacent ORF (ORF2) encodes a 39 kDa polyprotein which is expressed by leaky scanning mechanism *in vitro* and thought to be involved in the transmission of PCV by its vector. ORF2 starts 1 nt upstream of the first residue of UGA stop codon of the CP cistron. Further, downstream and separated by 135 nt intergenic region, is a triple gene block (TGB) that codes for proteins of 51, 14, and 17 kDa that are presumed to be involved in the cell to cell movement.

5′ and 3′ Noncoding Sequences

The 5′ and 3′ NCRs of RNA1 of pecluviruses are about 130 and 300 nt in length, respectively (Fig. 4), whereas the RNA2 has more diverse NCR of 390–500 nt at 5′ end and c. 300 nt at 3′ end. Within the 3′ NCR of RNA2, approximately 100 terminal nt are conserved among pecluvirus RNAs sequenced so far. The NCRs differ in size among isolates from the different serotypes. The 3′ NCR of pecluviruses, as in the case of furoviruses, forms a t-RNA-like structure (TLS) that aids in the replication of both RNAs.

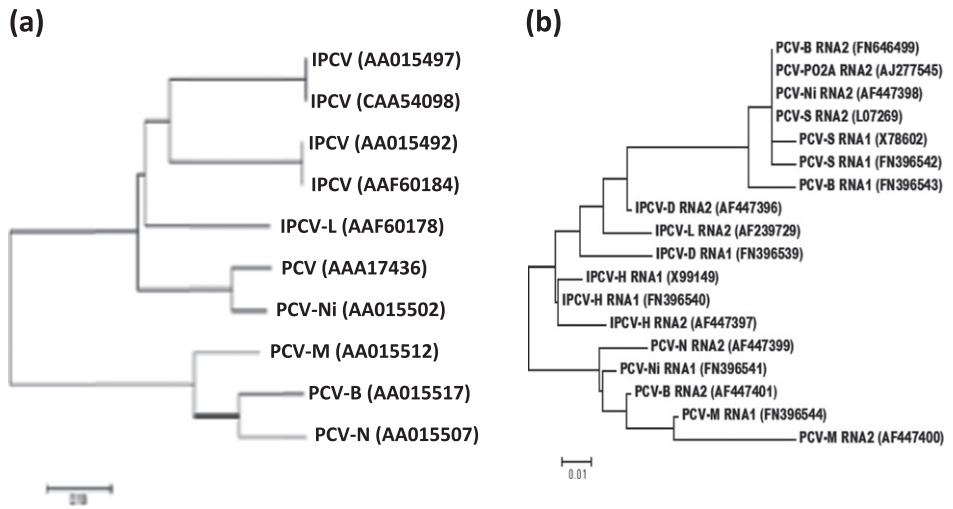

Fig. 5 (a) Phylogenetic tree constructed using coat protein (CP-P23) amino acid sequences from eight PCV/IPCV isolates. (b) Phylogenetic tree constructed using 18 sequences of the 3′ UTR of RNA1 and RNA2 of PCV/IPCV isolates. The evolutionary distances were computed using Maximum Composite Likelihood method by Tamura, K., Dudley, J., Nei, M. Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24, 1596-1599. GenBank Accession numbers are given in parentheses.

Sequence Comparisons and Phylogeny

Only one complete RNA1 genome sequence of each of PCV (X78602) and IPCV (X99149) are available, while five and three complete RNA2 sequences of PCV (L07269, AF447401, AF447400, AF447399, AF447398), and IPCV (AF447397, AF239729, AF447396), respectively are available in the database.

PCV and IPCV exhibited considerable differences in homology depending on the part of the genome sequence compared. The complete nt sequence of RNA1 of PCV is 78% similar to that of IPCV. The RNA1-encoded polypeptides (P191, P131, and P15) of PCV and IPCV share identities ranging from 75% (P15) to 95% (read-through portion of P191) and show significant similarities with furoviruses (e.g., 56% identity with polymerase of SBWMV). Comparison of the P15 gene shows a close relationship between IPCV isolates and a relatively high diversity among PCV isolates. The full-length sequence comparisons between RNA2 of PCV and IPCV isolates revealed a high degree of variability in size (between 58%–79%) and the proteins encoded by RNA2 are 39%–89% identical between species. Among the five ORFs of RNA2, ORF4 encoding P14 of TGB is highly conserved (90%–98%), whereas ORF2 encoded P39 is less conserved (25%–60%). Phylogenetic comparisons, based on complete RNA2 sequences, showed that the eight isolates could be grouped into three distinct clusters with no geographical distinction between PCV and IPCV isolates. Phylogenetic analysis of individual ORFs revealed an overall similarity with that obtained from complete RNA2 sequences, but the relative positions of individual isolates varied within each cluster. These studies indicate that there is substantial divergence among the RNA2's of pecluviruses and suggest that different polypeptides have evolved differently, possibly due to different selection pressures.

In the most members of the family *Virgaviridae*, CP is conserved among the isolates, but in the case of PCV and IPCV isolates, coat proteins are highly diverse (aa sequence identities between 37% and 89%) and have c. 30% similarity with the CP of BSMV (genus *Hordeivirus*). A conserved motif 'F-E-X_6-W' is present near the CP C-terminus of all three IPCV serotypes and PCV, as in the CPs of other rod-shaped viruses [*Tobacco mosaic virus* (TMV) and *Tobacco rattle virus* (TRV)]. Phylogenetic analysis of CP of eight PCV and IPCV isolates (PCV-B, PCV-M, PCV-N, PCV-Ni, PCV-S, IPCV-D, IPCV-H, IPCV-L) revealed that the PCV isolates grouped into two major clusters and IPCV into one cluster (**Fig. 5(a)**). The TGB proteins resemble those of *Potato mop top virus* (PMTV, genus *Pomovirus*).

Comparison of 3′ end nt sequences revealed that they are highly conserved (88% for RNA1 and 85% for RNA2), thus offering a potential target for broad-spectrum detection by RT-PCR. Conservation of 3′ UTR is verified by the alignment of eighteen 3′ UTR (124 nt long) sequences of RNA1 and RNA2 (collected from NCBI database) of PCV/IPCV isolates from different geographical regions and a phylogenetic tree has been constructed using the neighbor-joining method (**Fig. 5(b)**). The isolates were grouped into one Indian and two African clusters. Indian cluster appeared to be intermediate between the African clusters. Though there is high percentage of identity between 3′ UTR of RNA1 and RNA2 of PCV and IPCV isolates from different geographical regions, there is no evidence of reassortment between isolates. Putative recombination events and their frequencies in the complete genome of 168 accessions extracted from the International databases representing several members of six genera Viz. *Furovirus*, *Pecluvirus*, *Comovirus*, *Tobravirus*, *Hardeivirus*, and *Tobamovirus* indicate that exchange of heredity material occurred between non-homologous sequences like those of IPCV and PCV.

Assembly of Virus Particles

Sequences required for assembly of PCV virions have been established by testing the ability of encapsidation of deletion mutants of RNA1 and RNA2 *in vivo*. A putative origin of assembly (OAS) is mapped in the 5′ proximal part of the P15 gene of RNA1. Two sequence positions, 5′ proximal CP gene and the other in the p14 gene near the RNA2 3′ terminus that can drive encapsidation of RNA2, have been identified. No obvious sequence similarities between different assembly initiation sequences are noted.

The possible localization of an OAS in the CP gene of IPCV is realized as a result of the formation of virus-like particles (VLPs) in *Escherichia coli* and *N. benthamiana*, confirming the results observed for PCV. The monomer VLP size corresponded to the length of the encapsidated CP gene transcript RNA. Using immunocapture RT-PCR (IC-RT-PCR), such VLPs have been demonstrated to contain RNA encoding IPCV-H CP. Transgenic *N. benthamiana* expressing IPCV-H CP gene inoculated with the serologically distinct IPCV-L serotype accumulated virus particles that contained both types of CP, indicating that hetero-encapsidation occurs in transgenic plants.

Detection and Diagnosis

Pecluvirus diseased peanut plants occur in patches, mostly restricted to sandy/sandy-loam soils. The diseased peanut plants are stunted and dark green in color with or without chlorotic symptoms on leaves. Pecluvirus affected cereal and millet plants and grass weeds are difficult to recognize because they show mild or no visible symptoms. Diseases caused by pecluviruses reappear nearly in the same location year after year.

Immunoassays

Several immunological tests utilizing polyclonal (pAbs) and monoclonal antibodies (mAbs) have been used for the detection and identification of pecluvirus isolates. This approach revealed a diversity of serotypes and serogroups in Africa and India. The pAbs of PCV did not react with IPCV, BSMV, TMV, BNYVV, SBWMV, and PMTV. Wide serological diversity exists among PCV and IPCV isolates. Thus, serological tests have limitations to detect more than one isolate from a single antibody source. Antisera to different isolates of IPCV facilitated the grouping of isolates into three serotypes, IPCV-H (Hyderabad), IPCV-D (Durgapura), and IPCV-L (Ludhiana). All IPCV isolates are serologically distinct from PCV isolates and vice-versa. Utilizing a panel of mAbs raised against a PCV isolate and four different ELISA formats, several PCV isolates are grouped into five serotypes. Surprisingly, one of the mAbs reacted with IPCV-D in triple antibody sandwich ELISA. ISEM and alkaline phosphate or penicillinase-based ELISA formats have been applied for detection of both PCV and IPCV in various investigations.

Molecular Assays

It is essential to employ diagnostic tests that are highly sensitive and broadly specific for the detection of pecluviruses in disease surveys, to eliminate virus-contaminated sources in quarantine, and to devise strategies for disease control.

A broad-spectrum RT-PCR protocol, based on the conserved sequences of the 3′ end region of genomic and sub-genomic RNAs of several virus isolates, has been developed to detect all the currently known IPCV and PCV isolates in various plant samples originated from different countries. Primers are also designed for quantitation of pecluvirus isolates using a TaqMan-based real time RT-PCR (RT-qPCR). RT-PCR is compared with penicillinase-based DAS-ELISA for the detection of pecluvirus isolates in crop and weed plants. The virus is detected in 70% of suspected plants by RT-PCR as compared to 24% detected by DAS-ELISA. The RT-PCR enabled the detection of virus in the plants that did not exhibit overt symptoms. The detection and quantification of PCV and IPCV isolates (IPCV-L, IPCV-H, IPCV-D, PCV-B, PCV-M, PCV-N, PCV-S) is performed by RT-qPCR. These PCR-based techniques are very useful for detecting PCV and IPCV especially in non-symptomatic hosts. RT-qPCR is recognized as an indispensable tool for tracing resistant cultivars and for the maintenance of pecluvirus-free germplasm. Immunocapture RT-PCR has also been used to demonstrate the presence of RNA in PCV VLPs.

Vector

Separation of *P. graminis* resting spores from sorghum root material facilitated pAbs production. In DAC-ELISA, they detected one sporosorus per well of the ELISA plate. In spiked root samples, the procedure detected one sporosorus per mg of dried sorghum roots. The majority of *P. graminis* isolates from Europe, North America, and India reacted strongly with pAbs. However, *P. graminis* isolates from Rajasthan state (North India), from Pakistan, and an isolate from Senegal (West Africa) reacted weakly with pAbs. The DAC-ELISA procedure is applied to detect various stages in the life cycle of *P. graminis* and to detect infection that occurs under natural and controlled environments. Resting spores are detected in root sections by using fluorescein 5-isothiocyanate - labeled pAbs.

Probes specific for nuclear ribosomal DNA (rDNA) detected the presence of *P. graminis* f. sp. *subtropicalis*, *tropicalis*, and *temperata* in roots by RT-PCR. *P. graminis* f. sp. *tropicalis* is quantified in plants inoculated with viruliferous zoospores using a specific RT-qPCR. The ribotypes of *P. graminis* f. sp *temperata* and *P. graminis* f. sp. *tepida* are identified by restriction fragment length polymorphism analysis of PCR amplified rDNA, nested and multiplex PCR.

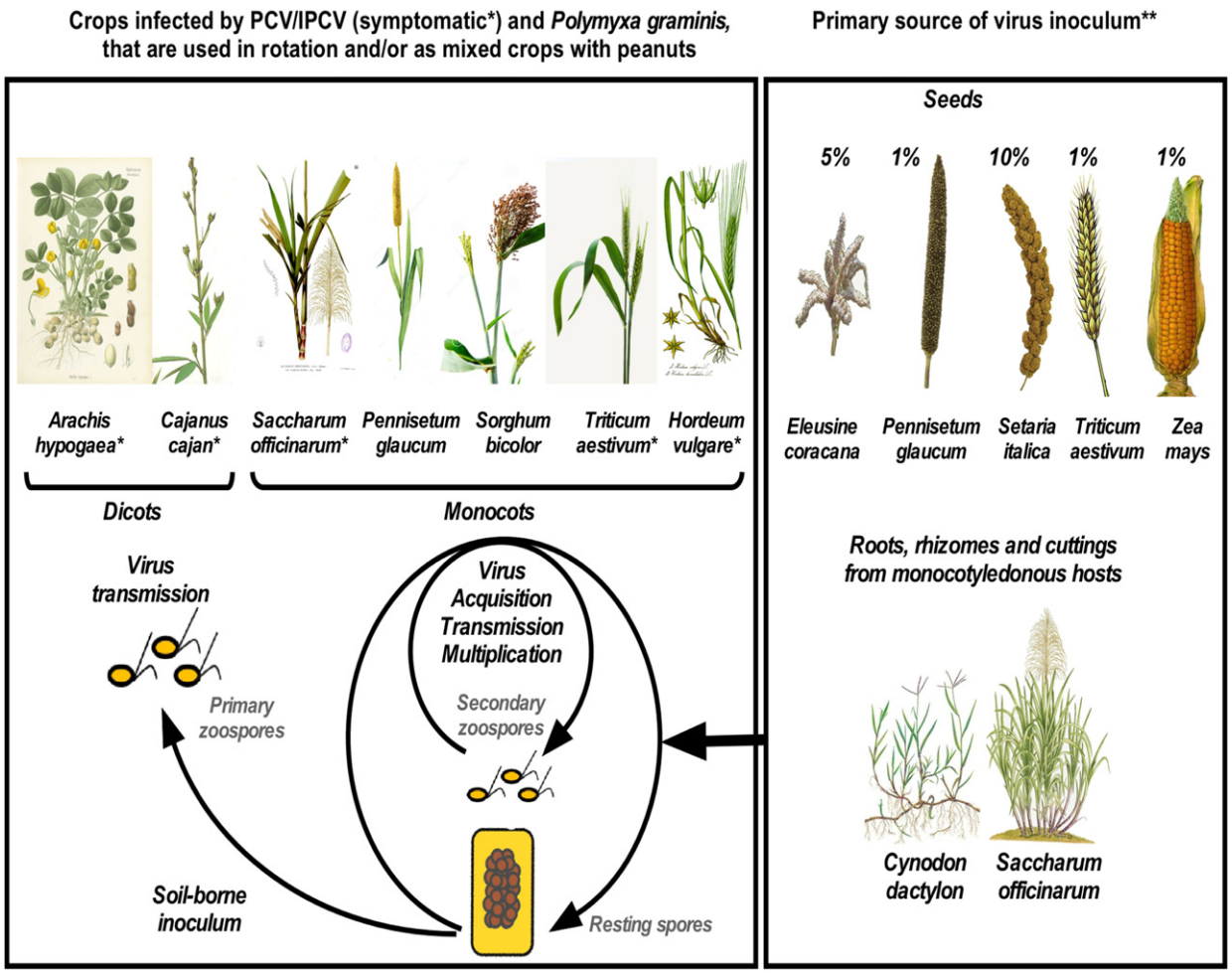

Fig. 6 Disease development cycle of Pecluviruses showing ways of acquisition, multiplication, and transmission by *Polymyxa graminis* through a variety of host plants. Modified from Delfosse P., 2000. Epidemiology and Management of the Indian peanut clump virus. Louvain-la-Neuve, Belgium: Université catholique de Louvain, PhD Thesis. Dieryck, B., Otto, G., Doucet, D., *et al.*, 2009. Seed, soil and vegetative transmission contribute to the spread of pecluviruses in West Africa and the Indian sub-continent. Virus Research 141, 184–189. Seed transmission data from Reddy, A.S., Hobbs, H.A., Delfosse, P., Murthy, A.K., Reddy, D.V.R., 1998. Seed transmission of Indian peanut clump virus (IPCV) in peanut and millets. Plant Disease 82, 343–346.

Epidemiology and Disease Cycle

The study of epidemiology and formulation of cost-effective disease control practices depend on the precise and sensitive detection of PCV and IPCV and their vector in plants, seeds, and soil. Peanut clump disease is largely confined to sandy soils and loams. Detection is required to identify infested fields, to select virus-free seed lots and vegetative propagules, to identify alternate hosts, and to assess the resistance of peanut, sugarcane and cereal and millet crops breeding lines.

Disease Development Cycle

The presence of viruliferous *P. graminis* vector, adequate soil moisture to facilitate the mobility of the vector zoospores from plant-to-plant in the sandy and sandy-loam fields, the optimum temperatures conducive to vector transmission and planting with seeds of virus-infected cereal crops contribute to primary spread. Presence of cereal hosts, ambient temperatures near 30°C and adequate moisture are vital for secondary spread. The relative contributions of favorable hosts for both the pecluvirus and its vector in the disease cycle are summarized (Fig. 6).

Dicotyledonous hosts limit the multiplication of the plasmodiophorid vector and hence considered them as fortuitous hosts that may not contribute to the perpetuation of the virus inoculum. Indeed, virus-infected peanut roots could not transmit or establish the disease. Monocotyledonous plants such as maize, sorghum, and pearl millet are 'preferred' hosts of the vector and contribute to the build-up of vector population potential in the soil. Seed of millets, maize and wheat, rhizomatous grasses (e.g., *C. dactylon*) and sugarcane vegetative propagules are likely to contribute to the disease establishment in new areas by supporting

the multiplication of the virus and vector. Soils from disease infested areas containing roots/rhizomes harbor resting spores of the vector. Dispersal of such infested soils by wind and displacement during tillering and plowing can contribute to the establishment of the disease in disease-free areas. During the rainy season, prolonged dry spells followed by supplementary irrigation in peanut fields may also contribute to the dispersal of resting spores and disease spread.

Disease Control

Research during the past four decades has led to the accumulation of considerable information on IPCV and PCV. It is now apparent that pecluviruses are not the viruses of peanut but should be regarded as viruses of cereals that opportunistically infect peanut. The difficulty in controlling such viruses is that they are both seed (in peanut, finger millet, foxtail millet, pearl millet, maize, wheat) and soil transmitted (through thick walled resting spores of the vector embedded in roots/rhizomes) and can also be propagated through sugarcane stem cuttings or the rhizomes of grass weeds. Continuous monocropping with fortuitous hosts such as peanut, bambara groundnut and pigeon pea will contribute to a reduction in plasmodiophorid population in soil. Seeds from infected cereal hosts should be avoided for planting. Thus, understanding precisely the sources of virus and vector and transmission mechanisms for each type of host plant in virus endemic agro-ecosystems using sensitive and specific diagnostic tools may led to formulation of effective disease control practices.

Initial experiments with IPCV in India showed that application of soil biocides, e.g., DD (a mixture of 1,3-dichloropropene and 1,2-dichloropropane), fumigant nematicides that also have fungicidal action and soil solarization are effective on a limited scale in decreasing the disease incidence in peanut fields. Clump affected peanut plants always occur in patches and easy to avoid. Additionally, under field conditions early infected, which is the case, hardly produce any seed.

Cultural Practices

The following crop cultural practices are suggested for reducing disease incidence:

- Early planting of peanut, before the onset of monsoons under judicious irrigation.
- Planting with trap crops such as pearl millet at a high density, plowing of two-week-old seedlings into the soil followed by planting with peanut seed.
- Avoid crop rotation and intercropping with cereal and millet crops.
- Adopt continuous cropping with dicotyledonous crops (peanut, bambara groundnut, cowpea, pigeon pea) for at least three successive growing seasons.

Host Plant Genetic Resistance

No resistance to IPCV is found in over 9000 cultivated *Arachis* germplasm. Unfortunately attempts to incorporate resistance to IPCV by genetic engineering, deploying CP gene, have not produced successful results. However, cultivation with cereal hosts that did not show any seed transmission may help in limiting the disease establishment at *hitherto* disease-free locations.

Future Perspectives

At present the geographical distribution of pecluviruses is restricted to the Indian subcontinent and West Africa. Locations where peanut crops are grown in rotation or as mixed crops with cereal crops on sandy soils can be regarded as targets for establishment of clump disease. Hence measures must be taken to avoid planting with seeds from cereal crops resourced from clump infested fields. They also should not be used towards germplasm exchange because they pose a quarantine risk of introduction of pecluviruses into new geographical regions. Mapping of locations with peanut crops grown in sandy soils in rotation with cereal crops and peanut crops intercropped with cereals, along with the temperature range required for infection, should help in determining the potential for occurrence of pecluviruses in new locations.

RNA interference (RNAi)-mediated resistance has been found to be highly effective in controlling RNA virus infections, and such approach needs to be exploited to produce pecluvirus-resistant crops. The suspected role of P39 in vector transmission of the pecluvirus needs to be confirmed by mutational analysis in order to deploy this ORF in transgenic research. The presence of sub-genomic RNAs encoding P14 and P17 in infected tissues needs to be verified. The cultural control measures tested for IPCV are worth exploiting in West Africa to minimize the impact of PCV. Development of duplex or multiplex RT-PCR is necessary to distinguish peanut clump and groundnut green rosette diseases that visually look alike. Broadly specific nonradioactive probes must be made available to researchers in Africa and India.

Further Reading

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