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BRIEF REPORT

Molecular diversity of poleroviruses infecting cucurbit crops in four countries reveals the presence of members of six distinct species

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Abstract When 66 cucurbit samples with yellowing symptoms from fields in Mali, the Philippines, Thailand and Uzbekistan were screened by RT-PCR using universal polerovirus primers, 21 were identified as harboring polerovirus RNA. When these 21 samples were screened with specific primers for the known cucurbit-infecting poleroviruses, suakwa aphid-borne yellows virus and a recombinant strain of cucurbit aphid-borne yellows virus were detected for the first time in the Philippines and Thailand. However, seven polerovirus-positive samples did not react with any of the known species-specific primers. Sequencing of 1.4-kb universal polerovirus RT-PCR products revealed the presence of two poleroviruses that had not been described previously. These viruses, from Mali and Thailand, were provisionally named pepo aphid-borne yellows virus and luffa aphid-borne yellows virus, respectively.

Currently, there are 13 formally accepted virus species in the genus *Polerovirus* (family *Luteoviridae*) [1]. Two of these, *Cucurbit aphid-borne yellows virus* (CABYV) and *Melon aphid-borne yellows virus* (MABYV), include viruses that infect economically important vegetable and fruit crops of the family Cucurbitaceae. Since the first identification of CABYV in 1992, CABYV has been detected worldwide infecting cucurbit plants [6, 7]. Recent molecular studies of cucurbit-infecting poleroviruses showed them to be relatively diverse by identifying genome sequences from MABYV, suakwa aphid-borne yellows virus (SABYV) and a recombinant CABYV strain (CABYV-R) with CABYV and MABYV as its most probable ancestors [4, 14]. In addition, the partial genome sequence of a polerovirus isolate from cucumber was deposited in GenBank as cucumber aphid-borne yellows virus Hainan isolate (CuABYV-HN, accession number FJ460218). Poleroviruses have positive-sense, single-stranded RNA genomes. The three 5' open reading frames (ORFs) (ORF0, ORF1 and ORF1+2) are separated by an intergenic region (IR) of about 200 nt from the 3' ORFs (ORF3, ORF4 and ORF3+5), which are expressed from a subgenomic RNA [1]. The IR is recognized as a hot spot for recombination in members of the family *Luteoviridae* [9]. The 5' half of the genomes of enamoviruses and poleroviruses differs fundamentally from that of luteoviruses. Luteoviruses lack an ORF0, and the replication related proteins encoded by ORFs 1 and 2 are more similar to those of members of the family *Tombusviridae*, whereas these ORFs from poleroviruses and enamoviruses are more similar to those of sobemoviruses [1]. In contrast to poleroviruses and luteoviruses, enamoviruses lacks an ORF4 [1]. The molecular species demarcation threshold within the genus *Polerovirus* is amino acid (aa) sequence identity of any gene product less than 90 % [1].

Diagnostic tools based on RT-PCR have been described that distinguish between CABYV, MABYV, SABYV and the CABYV common (CABYV-C) and recombinant (CABYV-R) strains [3]. However, with the exception of CABYV, all proposed cucurbit-infecting poleroviruses have so far only been found in China and Taiwan [3, 11, 14]. For future research and the development of cucurbit varieties with resistance to poleroviruses, it is important to

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be aware of the genetic diversity, incidence, and endurance or persistence of the different poleroviruses in different locations. To build a more complete picture of the distribution and diversity of cucurbit-infecting poleroviruses, cucurbit samples with yellowing symptoms from four countries (Mali, the Philippines, Thailand and Uzbekistan) were analyzed in this study.

RNA was extracted using a Plant Total RNA Miniprep Purification Kit (Hopegen, Taichung, Taiwan) according to the manufacturer's instructions from fresh leaf samples or leaf samples preserved in RNAfter^{TM} buffer (Hopegen, Taichung, Taiwan). Each sample was also tested for other common cucurbit-infecting viruses by enzyme-linked immunosorbent assay (ELISA) or RT-PCR (see Table 1). The samples from the Philippines were kindly provided by L. M. Dolores (Institute of Plant Breeding, University of the Philippines at Los Baños, Laguna, Philippines), and those from Uzbekistan where collected by Dr. Ravza Mavlyanova (AVRDC Regional Coordinator for Central Asia and the Caucasus).

In total, 66 cucurbit samples with diverse viral symptoms such as yellowing, vein-banding mosaic and shoestringing were studied. The samples from Mali and Uzbekistan (15 and 6, respectively) were screened by a one-step RT-PCR procedure with the general polerovirus primer pair Pol-G-F and Pol-G-R [3], whereas the samples from the Philippines and Thailand (18 and 27, respectively) were screened with the general polerovirus primer pair Gen1 and Gen2 [5]. Both tests were run as multiplex RT-PCR together with a primer pair amplifying the plant mitochondrial NADH dehydrogenase subunit 5 (*nad5*) gene as a control [8]. Of these 66 samples, 21 were identified by RT-PCR as infected with a polerovirus (Table 1). In a subsequent RT-PCR screening step, all 21 samples identified as infected with a polerovirus were tested for the presence of CABYV, MABYV and SABYV, and all CABYV-positive samples were then tested for the presence of the common or recombinant strain as described previously [3].

For samples that tested positive for the presence of a polerovirus but negative with the species-specific primers (and for a few representative samples that were positive with species-specific primers), a 1.4-kb portion of the polerovirus genome was amplified with the general polerovirus primer pair Pocon-F and Pocon-R in a two-step RT-PCR procedure and subsequently sequenced [3]. The 1.4-kb genome portion sequenced included the 3' half of the RNA-dependent RNA polymerase gene (RdRp, part of ORF2), followed by the IR and the coat protein (CP, ORF3) and movement protein (MP, ORF4) genes. The sequences obtained were subject to a BLASTn search and phylogenetic analysis to identify which polerovirus species each represented. Sequences were aligned, and phylogenetic trees were obtained by the neighbor-joining method with 100 bootstrap replications using CLUSTALX 1.83 [10] and viewed using TREEVIEW 1.5.2 [12].

The CABYV-C strain was detected in 11 of the 21 polerovirus-positive samples from the Philippines, Thailand and Uzbekistan. From Uzbekistan, all four polerovirus-positive samples were identified as CABYV-C (Table 1). CABYV-R was identified in one sample from the Philippines and one from Thailand. To our knowledge, this is the first report of detection of the recombinant strain outside Taiwan [3]. SABYV was identified in three samples from the Philippines and one sample from Thailand, and this represents the first description of SABYV outside China and Taiwan [3, 11]. Three samples were identified as carrying a mixed infection of CABYV and SABYV (Table 1). Mixed infections of different cucurbit-infecting poleroviruses were commonly detected in samples from China and Taiwan [3, 11, 14]. MABYV was not identified in any of the samples. However, four polerovirus-positive samples from Mali (ML3, ML4, ML7 and ML13) and three samples from Thailand (TH6, TH8 and TH24) were not amplified with any of the species-specific polerovirus primers.

The amplification products from 14 selected samples, obtained using the two-step RT-PCR protocol with the general polerovirus primer pair Pocon-F and Pocon-R [3], were cloned and sequenced. From these, 16 polerovirus contigs were assembled, and the sequences were submitted to GenBank (for accession numbers, see Table 1). The sequenced region covers the 3' half of the RdRp gene, followed by the IR and the CP and MP genes, thus allowing identification of members of the genus *Polerovirus*, family *Luteoviridae*, without a full-length genome sequence [3]. Phylogenetic analysis with other nucleotide sequences from GenBank showed the clustering of 10 isolates from this study with the respective reference sequences for CABYV-C (PH1, TH25, UZ2 and UZ4), CABYV-R (PH11 and TH18) and SABYV (PH11, PH12, PH16 and TH18), which was consistent with the results of the RT-PCR with species-specific primers (Fig. 1a and Table 1). However, the six contigs from polerovirus-positive samples that could not be identified by specific RT-PCR as CABYV, MABYV or SABYV formed two distinct phylogenetic clusters (Fig. 1a). One cluster comprised three isolates from Mali (ML3, ML7 and ML13), whereas the other cluster comprised three isolates from Thailand (TH6, TH8 and TH24) (Fig. 1a). To test if these two clusters represent new independent polerovirus species, further phylogenetic analysis of the deduced aa sequences for the partial RdRp, CP and MP was performed (Fig. 1b-d). The aa sequence identities within a cluster and between different clusters are shown in Table 2. From the samples identified as infected with CABYV-C, CABYV-R or SABYV by species-specific RT-PCR, the deduced aa sequences for the partial RdRp and CP regions showed greater than 90 % similarity

Table 1 Details of polerovirus-positive samples collected from Mali, the Philippines, Thailand and Uzbekistan

Country Cucurbit sp. (common name)	Province	Collection date	Sample code	Polerovirus detected[a]	Other viruses detected[b]
Mali					
Cucurbita pepo (summer squash)	Bamako	Jul 2008	ML3	PABYV (KF427699)	not determined
Cucurbita pepo (summer squash)	Bamako	Jul 2008	ML4	PABYV	not determined
Cucurbita pepo (summer squash)	Bamako	Jul 2008	ML7	PABYV (KF427700)	not determined
Cucumis melo (muskmelon)	Baguineda	Apr 2009	ML13	PABYV (KF427698)	ZYMV[c]
Philippines					
Cucumis sativus (cucumber)	Manila	May 2010	PH1	CABYV-C (HQ700823)	PV (ZYMV)
Cucumis sativus (cucumber)	Pangasinan	Jun 2010	PH6	CABYV-C	BV, PV
Cucumis melo (muskmelon)	Pangasinan	Jun 2010	PH8	CABYV-C	BV, PV
Luffa acutangula (angled luffa)	Pangasinan	Jun 2010	PH11	CABYV-R (HQ700824), SABYV (HQ700825)	
Luffa acutangula (angled luffa)	Pangasinan	Jun 2010	PH12	SABYV (HQ700826)	
Momordica charantia (bitter gourd)	Laguna	Nov 2010	PH14	CABYV-C	
Momordica charantia (bitter gourd)	Laguna	Nov 2010	PH15	CABYV-C	
Momordica charantia (bitter gourd)	Laguna	Nov 2010	PH16	CABYV-C, SABYV (HQ997896)	
Thailand					
Cucurbita moschata (pumpkin)	Ratchaburi	Sep 2008	TH6	LABYV (KF427702)	PRSV-W [d]
Luffa acutangula (angled luffa)	Ratchaburi	Sep 2008	TH8	LABYV (KF427703)	BV[d]
Coccinia grandis (ivy gourd)	Nakhon Pathom	Mar 2010	TH18	CABYV-R (KF427707), SABYV (KF427708)	PRSV-W[e]
Luffa acutangula (angled luffa)	Kanchanaburi	Jun 2010	TH24	LABYV (KF427701)	BV, PV (PRSV-W)[e]
Cucumis sativus (cucumber)	Nakhon Pathom	Jun 2010	TH25	CABYV-C (KF427704)	BV[e]
Uzbekistan					
Cucumis sativus (cucumber)	Tashkent	Aug 2009	UZ1	CABYV-C	CMV, PV (WMV)
Cucumis sativus (cucumber)	Tashkent	Aug 2009	UZ2	CABYV-C (KF427705)	CMV, PV (WMV)
Cucumis sativus (cucumber)	Tashkent	Aug 2009	UZ3	CABYV-C	
Cucumis melo (muskmelon)	Tashkent	Aug 2009	UZ4	CABYV-C (KF427706)	

[a] Poleroviruses analyzed by RT-PCR protocols described in the text; the GenBank accession numbers for a 1.4-kb sequence generated by RT-PCR with using general polerovirus primers Pocon-F and Pocon-R [3] are shown in parentheses

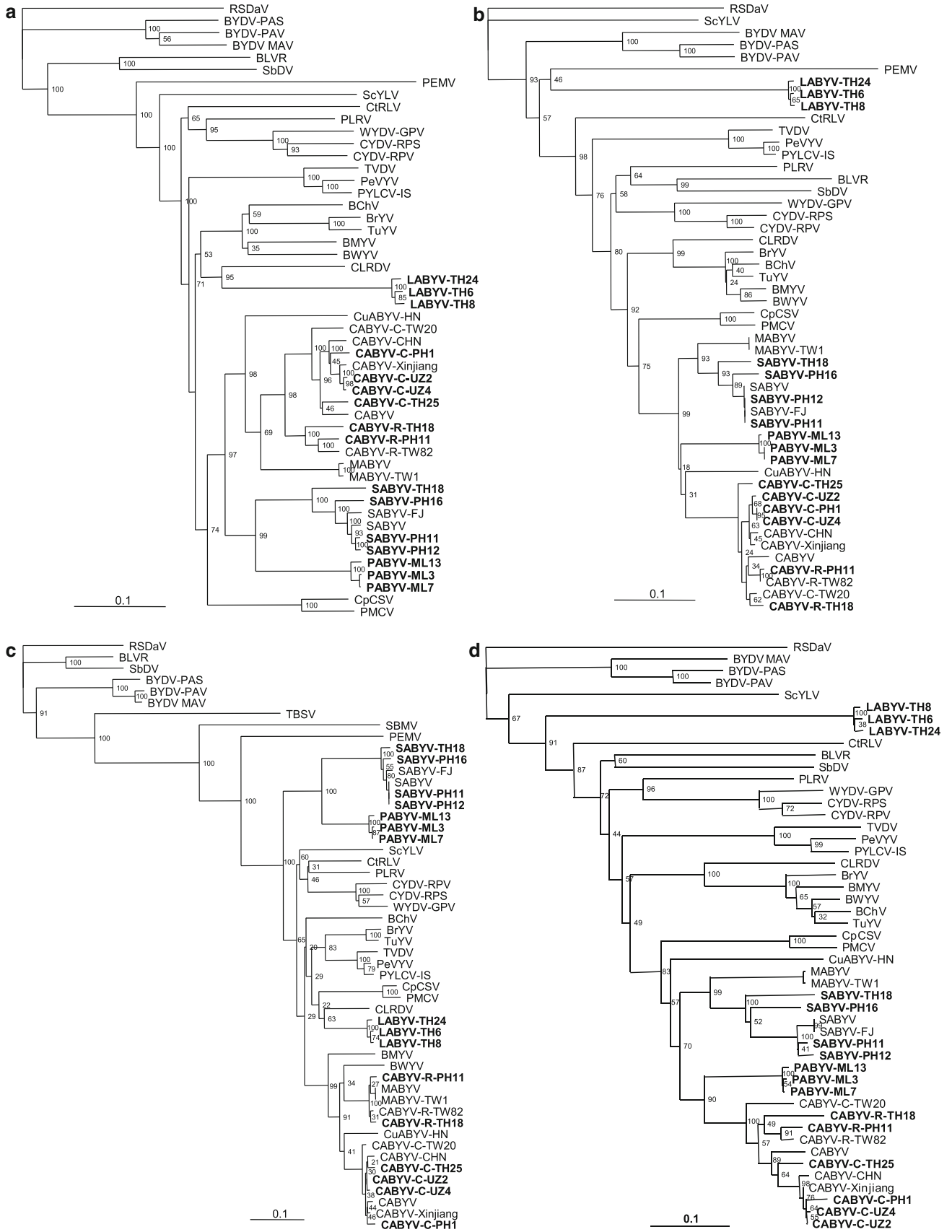
[b] Samples were tested by ELISA with antiserum against cucumber green mottle mosaic virus (CGMMV), cucumber mosaic virus (CMV), general tospovirus serogroup-4 watermelon silver mottle virus (TW-W), melon yellow spot virus (MYSV), papaya ringspot virus type W (PRSV-W), tomato spotted wilt virus (TSWV), watermelon silver mottle virus (WSMoV) and zucchini yellow mosaic virus (ZYMV) provided by S.D. Yeh, National Chung Hsing University, Taiwan; by ELISA with antiserum against watermelon mosaic virus (WMV; # AS-0203; DSMZ Braunschweig, Germany); by ELISA with general potyvirus antiserum (PV; # AS-0573/1; DSMZ Braunschweig, Germany); by PCR with universal begomovirus primer (BV) [13]; and by RT-PCR with specific cucurbit chlorotic yellows virus primer (CCYV) [2]

[c] Not tested for PV and CCYV

[d] Not tested for PV, TV-W, MYSV and WMV

[e] Not tested for TV-W

to the respective reference sequences in GenBank, whereas the deduced MP aa sequences were slightly less than 90 % similar to the respective MP reference sequences (Table 2). The sequence identities for deduced aa sequences for partial RdRp, CP and MP within the cluster of three sequences from Mali (ML3, ML7 and ML13) ranged from 97-100 % for each of the three proteins, but there was less than 90 % identity for all three proteins to any other members of the family *Luteoviridae* (Table 2). A similar result was obtained for the cluster of three sequences from Thailand (TH6, TH8, and TH24). The partial RdRp aa sequences of all isolates from the two new clusters were more similar to SBMV (33 % identity) than to TBSV (15-16 % identity) (Table 2), and since the sequences from these two clusters had an ORF4 (MP) region, each cluster was considered to represent a new polerovirus species. As two of the sequences from Mali were obtained from *Cucurbita pepo* (pumpkin or summer squash) samples, the virus represented by this cluster of sequences is provisionally named pepo aphid-borne yellows virus (PABYV). Similarly, the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LABYV-TH6
LABYV-TH8
CuABYV-HN
CABYV-C-TW20
CABYV-CHN
CABYV-C-PH1
CABYV-Xinjiang
CABYV-C-UZ2
CABYV-C-UZ4
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CABYV-R-PH11
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SABYV-FJ
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SABYV-PH12
PABYV-ML13
PABYV-ML3
PABYV-ML7
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CtRLV
PLRV
CYDV-RPV
CYDV-RPS
WYDV-GPV
BChV
BrYV
TuYV
TVDV
PeVYV
PYLCV-IS
CpCSV
PMCV
CLRDV
LABYV-TH24
LABYV-TH6
LABYV-TH8
BMYV
BWYV
CABYV-R-PH11
MABYV
MABYV-TW1
CABYV-R-TW82
CABYV-R-TH18
CuABYV-HN
CABYV-C-TW20
CABYV-CHN
CABYV-C-TH25
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PYLCV-IS
CLRDV
BrYV
BMYV
BWYV
BChV
TuYV
CpCSV
PMCV
CuABYV-HN
MABYV
MABYV-TW1
SABYV-TH18
SABYV-PH16
SABYV
SABYV-FJ
SABYV-PH11
SABYV-PH12
PABYV-ML13
PABYV-ML3
PABYV-ML7
CABYV-C-TW20
CABYV-R-TH18
CABYV-R-PH11
CABYV-R-TW82
CABYV
CABYV-C-TH25
CABYV-CHN
CABYV-Xinjiang
CABYV-C-PH1
CABYV-C-UZ4
CABYV-C-UZ2
0.1

◀ **Fig.1** Phylogenetic tree based on alignment of members of the genera *Luteovirus*, *Polerovirus* and *Enamovirus* using (**a**) a partial polerovirus genome nucleotide sequence (1.4 kb) spanning from the 3'-end partial RdRp (P2) to the coat protein (P3) generated with the general polerovirus primer pair Pocon-F and Pocon-R [3], (**b**) aa sequences of the CP, (**c**) aa sequences of the 3'half of the RdRp, and, (**d**) aa sequences of the of the MP. Bootstrap values (%) are shown at nodes. Virus names, abbreviations and GenBank accession numbers used for this alignment are as follows: barley yellow dwarf virus-MAV (BYDV-MAV, NC_003680), barley yellow dwarf virus-PAS (BYDV-PAS, NC_002160), barley yellow dwarf virus-PAV (BYDV-PAV, NC_004750), bean leafroll virus (BLRV, NC_003369), beet chlorosis virus (BChV, NC_002766), beet mild yellowing virus (BMYV, NC_003491), beet western yellows virus (BWYV, NC_004756), brassica yellows virus (BrYV, NC_016038), carrot red leaf virus (CtRLV, NC_006265), cereal yellow dwarf virus-RPS (CYDV-RPS, NC_002198), cereal yellow dwarf virus-RPV (CYDV-RPV, NC_004751), chickpea chlorotic stunt virus (CpCSV, NC_008249), cotton leafroll dwarf virus (CLRDV, NC_014545), cucumber aphid-borne yellows virus isolate Hainan (CuABYV-HN, FJ460218), cucurbit aphid-borne yellows virus (CABYV, NC_003688), cucurbit aphid-borne yellows virus common strain isolate Taiwan-20 (CABYV-C-TW20, JQ700305), cucurbit aphid-borne yellows virus isolate China (CABYV-CHN, EU000535), cucurbit aphid-borne yellows virus isolate Xinjiang (CABYV-Xinjiang, EU636992), cucurbit aphid-borne yellows virus recombinant strain isolate Taiwan-82 (CABYV-R-TW82, JQ700306), melon aphid-borne yellows virus isolate Taiwan-1 (MABYV-TW1, JQ700307), melon aphid-borne yellows virus (MABYV, NC_010809), pea enation mosaic virus (PEMV, NC_003629), pea mild chlorosis virus (PMCV, JF507725), pepper vein yellows virus (PeVYV, NC_015050), pepper yellow leaf curl virus Israel strain (PYLCV-IS, HM439608), potato leafroll virus (PLRV, NC_001747), rose spring dwarf-associated virus (RSDaV, NC_010806), southern bean mosaic virus (SBMV, NC_004060), soybean dwarf virus (SbDV, NC_003056), suakwa aphid-borne yellows virus isolate Fujian (SABYV-FJ, FJ425878), suakwa aphid-borne yellows virus (SABYV, NC_018571), sugarcane yellow leaf virus (ScYLV, NC_000874), tobacco vein distorting virus (TVDV, NC_010732), tomato bushy stunt virus (TBSV, NC_001554), turnip yellows virus (TuYV, NC_003743), and wheat yellow dwarf virus-RPV (WYDV-GPV, NC_012931)

virus represented by the sequences from the samples from Thailand is provisionally named luffa aphid-borne yellows virus (LABYV), since two of these samples were from *Luffa acutangula* (ridged or angled luffa). The sequence in GenBank named "cucumber aphid-borne yellows virus Hainan isolate" (CuABYV-HN, FJ460218) (not generated in this study and without a corresponding publication) was included in the sequence and phylogenetic analysis outlined above. This sequence also fulfilled all the criteria necessary to be considered as representing a distinct species of cucurbit-infecting polerovirus (Table 2). However, CuABYV is so far only represented by one sequence from a single sample, whereas PABYV and LABYV sequences were obtained from several independent samples from different years (Table 1).

For the newly identified poleroviruses, PABYV and LABYV, diagnostic RT-PCR protocols were developed. For the detection of PABYV the primer PABYV-456-F (5'-AGCACTATACCAGCAAACAGAAAACG-3') was developed, which, when used in combination with Pol-G-R [3], yields a product of 456 bp if PABYV is present. To test for LABYV, the primer pair LABYV-225-F (5'-CA-ATCATGGTCCTCCCTAACTT-3') and LAB-R (5'-TCTTATAGCGAGCGTAGGACTTGAG-3') was developed. In RT-PCR (63 °C annealing temperature and 40 s extension time), these yield a 225-bp product if LABYV is present. Both RT-PCR tests can be run multiplexed with a primer pair amplifying the plant mitochondrial *nad5* gene as an internal control [8]. All 21 samples identified as polerovirus-infected were re-screened with the new primer sets to detect PABYV and LABYV, and this confirmed the presence of PABYV in sample ML4, but LABYV was not detected in any other samples.

Here, we have shown the great diversity of cucurbit-infecting poleroviruses by describing members of six distinct species according to phylogenetic analysis and the current classification criteria. The current International Committee on Taxonomy of Viruses species demarcation threshold for poleroviruses is an aa sequence difference in any gene product of more than 10 %. Since differences greater than 10 % were observed in the MP aa sequences within the groups identified as CABYV-C, CABYV-R and SABYV, it could be argued that each of these should be split to form further new species. However, since the aa sequence differences in both the CP and the partial RdRp gene products analyzed here were less than 10 %, and for consistency with previous publications, we do not propose to subdivide these groups.

The CABYV common strain was identified in 11 of the 21 positive polerovirus samples. CABYV-C was detected in samples from the Philippines, Thailand and Uzbekistan, and sequence information for each country is stored in the GenBank database. MABYV was not detected in any of the four countries in this study. This may be attributed to the relatively small total number of samples analyzed and the small proportion from melon crops. In a comparable study from Taiwan using the same sampling and analysis methods, out of 29 polerovirus-positive samples from a starting set of 102 cucurbit samples showing virus-like symptoms, MABYV was detected in three watermelon (*Citrullus lanatus*), three wax gourd (*Benincasa hispida*), and one bottle gourd (*Lagenaria siceraria*) sample [3]. Here, we conclude that SABYV and CABYV-R are more widespread in Southeast Asia by presenting for the first time sequence evidence of their occurrence outside China in the Philippines and Thailand, though not in Mali or Uzbekistan . In Mali, we detected only the previously undescribed polerovirus, PABYV, though it should be noted that only a few samples were available for testing. So far, little is known about the biological significance of the different

Table 2 Amino acid sequence identities (%) between three polerovirus proteins and homologous proteins from related viruses

Protein Virus	Virus											
	CABYV-C[a]	CABYV-R[b]	MABYV[c]	SABYV[d]	PABYV[e]	LABYV[f]	CuABYV	Polerov.[g]	Luteov.[h]	PEMV	SBMV	TBSV
RdRp-par[i]												
CABYV-C[a]	96-100	87-89	87-88	64-65	65-66	75-76	87-90	67-87	16-20	49-50	33-34	13-14
CABYV-R[b]		97-100	98	62-64	65-66	73-74	83-85	66-87	16-21	48-49	36	13
MABYV[c]			100	63-64	65	73-74	85	67-87	16-20	49	36	14
SABYV[d]				95-100	77-79	63-65	63-65	59-67	15-16	47-48	31-32	11-12
PABYV[e]					98-100	67-69	65-66	63-71	15-17	48	33	15
LABYV[f]						97-100	73-74	68-83	15-19	49	33	15-16
CuABYV							100	69-87	16-18	50	34	14
Coat protein												
CABYV-C[a]	94-100	94-97	80-82	79-83	78-83	42-43	80-83	38-71	36-58	32-33		
CABYV-R[b]		96-100	80-82	79-82	79-81	42	82-83	38-72	36-58	32		
MABYV[c]			100	86-89	81	41	81	40-73	37-57	31		
SABYV[d]				90-100	79-82	41-42	81-83	39-72	39-57	30-31		
PABYV[e]					99-100	41-42	80	36-70	36-60	29		
LABYV[f]						98-100	42	32-42	32-43	30		
CuABYV							100	39-73	40-57	30		
Movement protein												
CABYV-C[a]	84-100	83-90	64-70	64-70	76-79	25-28	61-66	22-61	20-52			
CABYV-R[b]		88-100	69-71	64-70	74-76	26-27	63-66	25-61	20-49			
MABYV[c]			99-100	74-77	71-72	27-28	63-64	31-57	21-46			
SABYV[d]				82-100	69-73	23-26	64-65	28-59	19-46			
PABYV[e]					98-100	27-28	65	27-60	19-45			
LABYV[f]						98-100	26-27	18-26	19-30			
CuABYV							100	29-57	17-49			

Full virus names are given in Table 1, and the GenBank accession numbers of the sequences used are shown in Table 1 and Fig. 1

[a] CABYV-C: CABYV common strain represented by 8 isolates; CABYV, CABYV-CHN, CABYV-C-PH1, CABYV-C-TH25, CABYV-C-TW20, CABYV-C-UZ2, CABYV-C-UZ4 and CABYV-Xinjiang

[b] CABYV-R: CABYV recombinant strain represented by 3 isolates; CABYV-R-PH11, CABYV-R-TH18 and CABYV-R-TW82

[c] MABYV: MABYV species represented by 2 isolates; MABYV and MABYV-TW1

[d] SABYV: SABYV species represented by 6 isolates; SABYV, SABYV-FJ, SABYV-PH11, SABYV-PH12, SABYV-PH16 and SABYV-TH18

[e] PABYV: PABYV species represented by 3 isolates; PABYV-ML3, PABYV-ML7 and PABYV-ML13

[f] LABYV: LABYV species represented by 3 isolates; LABYV-TH6, LABYV-TH8 and LABYV-TH24

[g] Polerov.: *Polerovirus* genus represented by 17 species: BChV, BMYV, BrYV, BWYV, CLRDV, CpCSV, CtRLV, CYDV-RPS, CYDV-RPV, PeVYV, PLRV, PMCV, PYLCV-IS, ScYLV, TuYV, TVDV and WYDV-GPV

[h] Luteov.: *Luteovirus* genus represented by 6 species: BYDV-MAV, BYDV-PAS, BYDV-PAV, BLVR, RSDaV and SbDV

[i] RdRp-par: RdRp partial aa sequence compared, start from aa 865 of the replicase fusion protein (ORF1 and ORF2) (reference CABYV-FRA)

cucurbit-infecting poleroviruses, and serology-based diagnostics are not available to distinguish between these viruses. The RT-PCR diagnostic methods developed in this study will be useful in this respect, since for breeding and selection of virus-resistant plants, it is important to know which viruses and strains are prevalent in each target region and crop species.

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