Respiratory syncytial virus in adult patients at a tertiary care hospital in Germany: clinical features and molecular epidemiology of the fusion protein in the severe respiratory season of 2022/2023

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Supplementary Material

Table S1. Primers used for RSV (F gene) sequencing.

Primer	Sequence (5´ to 3´)
RSV-A-F-FNA	TCCACTCAACCACCTCCGAA
RSV-A-F-FNI1	ACATCCGAGTACCTATCACAATCT
RSV-A-F-FNI2	AGACAGCAAAGTTACTCTATCATGTC
RSV-A-F-RNA	TCCAACTCTGCAGCTCCACT
RSV-A-F-RNI1	TACCATCCTCTGTCGGTTCTTG
RSV-A-F-RNI2	TGTCTTACAAGCAGTGCATGGG
RSV-B-F-FNA	ACACCAGCACCTCACAATCCA
RSV-B-F-FNI1	CACCCAACTCCACACAAACAC
RSV-B-F-FNI2	CAGTTATAGAATTCCAGCAGAAGAAC
RSV-B-F-RNA	TGCTCTTGTCCATTGACTTGAGTAT
RSV-B-F-RNI1	AGCAAGGTGTATCAATTACACCATAGAT
RSV-B-F-RNI2	GGCCATTCAAAGTAATTATGACTGTAG

Primer concentration: 10 pmol/µl. The primers were designed for the current study.

Table S2. Reagent composition for RSV-A sequencing (first PCR).

	Concentration	Volume [µl]	Final concentration
H_20		20	
5xBuffer	5x (+12,5mM $MgCl_2$)	10	1x (+2,5mM $MgCl_2$)
Primer 1	10 pmol/µl	3	30 pmol
Primer 2	10 pmol/µl	3	30 pmol
dNTP's	10 mM each	1	200 µM
Enzyme-Mix		2	
RNase Inhibitor	40 Units/µl	1	40 Units
RNA		10	

Reagent concentrations and volumes used for RSV-A sequencing (F gene). The QIAGEN® OneStep RT-PCR Kit (Cat.No.210212) was used. Two separate nested PCRs with different primer sets were performed for the amplification of two fragments: RSV-A-F-FNA and RSV-A-F-RNI1 (set 1) and RSV-A-F-FNI2 and RSV-A-F-RNA (set 2).

Table S3. Reagent composition for RSV-B sequencing (first PCR).

	Concentration	Volume [µl]	Final concentration
H20		20	
5xBuffer	5x (+12,5mM $MgCl_2$)	10	1x (+2,5mM $MgCl_2$)
Primer 1	10 pmol/µl	3	30 pmol
Primer 2	10 pmol/µl	3	30 pmol
dNTP's	10 mM each	1	200 µM
Enzyme-Mix		2	
RNase Inhibitor	40 Units/µl	1	40 Units
RNA		10	

Reagent concentrations and volumes used for RSV-B sequencing (F Gene). The QIAGEN® OneStep RT-PCR Kit (Cat.No.210212) was used. Two separate nested PCRs with different primer sets were performed for the amplification of two fragments: RSV-B-F-FNA and RSV-B-F-RNI1 (set 1) and RSV-B-F-FNI2 and RSV-B-F-RNA (set 2).

Table S4. Cycling conditions for RSV-Sequencing (first PCR).

Reaction	Temperature	Time	Cycles
Reverse transcription	50°C	30 min	1
Enzyme activation	95°C	15 min	1
Denaturation	94°C	1 min	45
Annealing	56°C	30 s	
Elongation	72°C	1 min 30 s	
Final elongation	72°C	5 min	1
Cooling	4°C	forever	

The amplification was performed with a GeneTouch Thermal Cycler BTC33BAS (GeneTouch Corp., Taoyuan City, Taiwan).

Table S5. Reagent composition for RSV-A sequencing (nested PCR).

	Concentration	Volume [µl]	Final concentration
H_20		33,1	
10xBuffer,-$MgCl_2$	10x	5	1x
$MgCl_2$	50 mM	1,5	1,5 mM
Primer 1	10 pmol/µl	2	20 pmol
Primer 2	10 pmol/µl	2	20 pmol
dNTP's	10 mM each	1	200 µM
Platinum Taq	5 Units/µl	0,4	2 Units
First round PCR-product		5	

Reagent concentrations and volumes used for RSV-A sequencing (F gene). The Invitrogen Platinum™ II Taq DNA Polymerase (Cat.No. 10966034) was used. Two separate nested PCRs with different primer sets were performed for the amplification of two fragments: RSV-A-F-FNI1 and RSV-A-F-RNI1 (set 1) and RSV-A-F-FNI2 and RSV-A-F-RNI2 (set 2).

Table S6. Reagent composition for RSV-B sequencing (nested PCR).

	Concentration	Volume [µl]	Final concentration
H_20		33,1	
10xBuffer,-$MgCl_2$	10x	5	1x
$MgCl_2$	50 mM	1,5	1,5 MM
Primer 1	10 pmol/µl	2	20 pmol
Primer 2	10 pmol/µl	2	20 pmol
dNTP's	10 mM each	1	200 µM
Platinum Taq	5 Units/µl	0,4	2 Units
First round PCR-product		5	

Reagent concentrations and volumes used for RSV-A sequencing (F gene). The Invitrogen Platinum™ II Taq DNA Polymerase (Cat.No. 10966034) was used. Two separate nested PCRs with different primer sets were performed for the amplification of two fragments: RSV-B-F-FNI1 and RSV-B-F-RNI1 (set 1) and RSV-B-F-FNI2 and RSV-B-F-RNI2 (set 2).

Table S7. Cycling conditions for Sanger Sequencing (nested PCR).

Reaction	Temperature	Time	Cycles
Enzyme activation	95°C	1 min	1
Denaturation	94°C	30 s	45
Annealing	56°C	30 s	
Elongation	72°C	1 min 20 s	
Final elongation	72°C	5 min	1
Cooling	4°C	forever	

The amplification was performed on a GeneTouch Thermal Cycler BTC33BAS (GeneTouch Corp., Taoyuan City, Taiwan). The resulting PCR products were used for Sanger sequencing.

Table S8. RSV-A Reference Sequences Goya et al.

GA1	AY911262*	GA2.3.3	KY654511	GA3.0.0	KU316149
GA1	JX198138	GA2.3.3	MF001041	GA3.0.0	MG642074
GA1	KJ723474	GA2.3.3	MF001047	GA3.0.1	KP258699
GA1	KU316165	GA2.3.3	MF001054	GA3.0.1	KU316133
GA2	MG642063	GA2.3.4	JF920053	GA3.0.1	MG642031
GA2.1	KJ723483	GA2.3.4	KC731483	GA3.0.2	KJ723465
GA2.1	KP258723	GA2.3.4	KJ672455	GA3.0.2	KP258701
GA2.1	KU316098	GA2.3.4	KJ672482	GA3.0.2	KP258726
GA2.1	MG642070	GA2.3.4	KP663728	GA3.0.2	KU316104
GA2.2	JF920062	GA2.3.4	KU950667	GA3.0.2	KU316161
GA2.2	JX069801	GA2.3.4	KX655658	GA3.0.2	KU316170
GA2.2	KJ723492	GA2.3.4	KX765920	GA3.0.2	MG642048
GA2.2	KP258700	GA2.3.4	KY460517	GA3.0.3a	JQ901455
GA2.2	KP258743	GA2.3.4	KY654508	GA3.0.3a	JX069802
GA2.2	KU316092	GA2.3.4	MF001051	GA3.0.3a	KF826847
GA2.2	MG642030	GA2.3.4	MF001053	GA3.0.3a	KM360090
GA2.3.0	JX069798	GA2.3.5	KJ672467	GA3.0.3a	KY967364
GA2.3.0	KP119748	GA2.3.5	KJ672470	GA3.0.4a	KF530260
GA2.3.0	KU316118	GA2.3.5	KT285064	GA3.0.4a	KF826854
GA2.3.0	KU950573	GA2.3.5	KU950506	GA3.0.4b	KF826826
GA2.3.0	MG642033	GA2.3.5	KU950531	GA3.0.4b	KF826827
GA2.3.1	JQ901452	GA2.3.5	KU950540	GA3.0.4b	KF826850
GA2.3.1	JX015480	GA2.3.5	KU950550	GA3.0.4b	KF973333
GA2.3.1	KJ627305	GA2.3.5	KU950556	GA3.0.5b	KF826832
GA2.3.2b	JX015486	GA2.3.5	KU950560	GA3.0.5b	KX765933
GA2.3.2b	KJ627284	GA2.3.5	KU950596	GA3.0.5b	MF001038
GA2.3.2b	KJ627336	GA2.3.5	KU950650		
GA2.3.3	JX015482	GA2.3.5	KU950651		
GA2.3.3	JX015491	GA2.3.5	KU950670		
GA2.3.3	JX015497	GA2.3.5	KU950692		
GA2.3.3	KF826838	GA2.3.5	KX765917		
GA2.3.3	KF826855	GA2.3.5	KX765932		
GA2.3.3	KJ627256	GA2.3.5	KX765941		
GA2.3.3	KJ627294	GA2.3.5	KX765954		
GA2.3.3	KJ627320	GA2.3.5	KX765971		
GA2.3.3	KJ627337	GA2.3.5	KX894807		
GA2.3.3	KJ627338	GA2.3.5	KY654514		
GA2.3.3	KJ627349	GA2.3.5	KY654518		
GA2.3.3	KJ627370	GA2.3.5	KY883567		
GA2.3.3	KJ939951	GA2.3.5	MG773271		
GA2.3.3	KP317953	GA3.0.0	KJ723486		
GA2.3.3	KX655662	GA3.0.0	KP258709		
GA2.3.3	KX655672	GA3.0.0	KP258715		
GA2.3.3	KX765958	GA3.0.0	KU316137		

Accession numbers of proposed genotypes [16]. *This sequence was used as RSV-A prototype strain sequence for the rooting of the phylogenetic trees throughout the manuscript.

Table S9. RSV-B Reference Sequences Goya et al.

GB1	AY353550*	GB5.0.3	MG431252
GB1	JX198143	GB5.0.4a	KJ939929
GB1	KP258736	GB5.0.4a	KJ939932
GB2	AF013254	GB5.0.4a	KU950467
GB2	JX198165	GB5.0.4a	KX655648
GB2	KP258712	GB5.0.4a	KX655653
GB2	KU316127	GB5.0.4a	KX765912
GB2	KU316173	GB5.0.4a	KX765957
GB2	KU316175	GB5.0.4a	KX765962
GB2	KU316181	GB5.0.4a	KY249657
GB2	KU316182	GB5.0.4a	KY249670
GB2	MG642036	GB5.0.4a	KY249677
GB2	MG642043	GB5.0.4a	KY883571
GB3	JX198147	GB5.0.4b	JN032115
GB3	JX198166	GB5.0.4b	JX576730
GB4	JX198160	GB5.0.4b	JX576746
GB4	MG642062	GB5.0.4b	JX576751
GB5.0.0	JX576760	GB5.0.4b	KF826860
GB5.0.0	KF826853	GB5.0.4b	KJ627285
GB5.0.0	KP258713	GB5.0.4c	KJ627262
GB5.0.0	KP258724	GB5.0.4c	KJ939928
GB5.0.0	KP317923	GB5.0.4c	KP317928
GB5.0.0	KU316134	GB5.0.4c	KU950477
GB5.0.0	KU316179	GB5.0.4c	KU950588
GB5.0.0	MF185754	GB5.0.4c	KX655649
GB5.0.1	JX576761	GB5.0.4c	KX655654
GB5.0.1	JX576762	GB5.0.4c	KX765949
GB5.0.1	KF826829	GB5.0.4c	KY249658
GB5.0.1	KJ939919	GB5.0.4c	MG431251
GB5.0.1	MF185752	GB5.0.5a	KX765906
GB5.0.2	JX576742	GB5.0.5a	KY249683
GB5.0.2	KF826843	GB5.0.5a	KY684758
GB5.0.2	KF826845	GB5.0.5a	MG773268
GB5.0.2	KJ627302	GB5.0.5a	MG839547
GB5.0.2	KJ939926	GB6	MF185751
GB5.0.2	KU950484		
GB5.0.2	KU950619		
GB5.0.2	KX655669		
GB5.0.2	KX765943		
GB5.0.2	KY249662		
GB5.0.3	JN032117		
GB5.0.3	JX576744		
GB5.0.3	KF826839		
GB5.0.3	KU950458		

Accession numbers of proposed genotypes [16]. *This sequence was used as RSV-B prototype strain sequence for the rooting of the phylogenetic trees throughout the manuscript.

Table S10. Amino acid residues of antigenic sites Ø – V.

Antigenic site	Footprints	Residues [n]	Residues
Site Ø	5C4	26	63, 64, 65, 66, 67, 68, 69, 77, 83, 168, 196, 197, 198, 200, 201, 202, 204, 205, 206, 207, 208, 209, 211, 212, 294, 295
	D25	28	62, 63, 64, 65, 66, 67, 68, 69, 71, 72, 73, 74, 83, 197, 198, 200, 201, 202, 204, 205, 206, 207, 208, 209, 210, 211, 212, 216
	5C4 + D25	33	62, 63, 64, 65, 66, 67, 68, 69, 71, 72, 73, 74, 77, 83, 168, 196, 197, 198, 200, 201, 202, 204, 205, 206, 207, 208, 209, 210, 211, 212, 216, 294, 295
Site I	ADI-14349	20	31, 32, 33, 34, 35, 40, 42, 43, 312, 313, 314, 344, 377, 378, 380, 381, 383, 384, 389, 390
Site II	Motavizumab	20	65, 95, 176, 255, 258, 259, 261, 262, 263, 267, 268, 269, 271, 272, 275, 276, 309, 310, 363, 364
Site III	MPE8	50	45, 50, 51, 52, 53, 54, 150, 178, 180, 185, 186, 187, 188, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 276, 277, 305, 306, 307, 309, 310, 311, 312, 344, 345, 346, 347, 364, 377, 425, 427, 428, 429, 430, 431, 448, 449, 456, 458
Site IV	101F	30	50, 416, 418, 419, 420, 421, 422, 423, 425, 426, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 440, 446, 450, 451, 452, 453, 454, 455, 456, 457
Site V	hRSV90	21	169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 188, 191, 194, 196, 197, 200, 201, 226, 262, 263, 271

The antigenic sites were investigated as described in Mas et al. [25]. Residues that are located on the neighboring protomer are underlined.

Table S11. Co-infecting pathogens.

Bacteria	*n*	Viruses	*n*	Fungi	*n*
Pseudomonas aeruginosa	9	Influenza A H3N2	16	*Aspergillus fumigatus*	2
Klebsiella pneumoniae	8	SARS-CoV-2	12	*Aspergillus niger*	2
Streptococcus pneumoniae	6	Parainfluenzavirus Type 3	6	*Pneumocystis jirovecii*	*2*
Staphylococcus aureus	5	Rhinovirus	5		
Escherichia coli	3	Metapneumovirus	3		
Serratia marcescens	3	CMV	2		
Haemophilus influenzae	2	Coronavirus OC43	2		
Enterobacter cloacae	2	Influenza B	2		
Chlamydophila pneumoniae	1	VZV	1		
Enterococcus faecium	1	Influenza A H1N1	1		
Haemophilus parahaemolyticus	1	Adenovirus	1		
Proteus mirabilis	1	Parainfluenzavirus Type 1	1		
Raoultella ornithinolytica	1	HSV 1	1		
Stenotrophomonas maltophilia	1	Dengue Virus	1		

Pathogens detected by type, with n showing their frequencies of detection. The co-infections included cases with detections of more than one pathogen.

Table S12. Study population and clinical features of season 2021/2022 and season 2022/2023 RSV cases.

		2021/2022	2022/2023	total	*p*-value
Study population					
Female	[% (*n*/total)]	42.3 (11/26)	47.9 (70/146)	47.1 (81/172)	n.s.
Male	[% (*n*/total)]	57.6 (15/26)	52.1 (76/146)	52.9 (91/172)	
Age [years]	[median (IQR)]	49.5 (31.5 – 64.25)	65 (49.75 – 78)	64 (46 – 75.75)	**<0.001**
Inpatients	[% (*n*/total)]	76.0 (19/25)	82.8 (120/145)	81.8 (139/170)	n.s.
Outpatients	[% (*n*/total)]	24.0 (6/25)	17.2 (25/145)	18.2 (31/170)	
Length of hospital stay [days]	[median (IQR)]	12 (6.5 – 28)	10 (4 – 20)	10.5 (4.74 – 19.25)	n.s.
Comorbidities and risk factors					
Obstructive lung disease [OLD]	[% (*n*/total)]	20.8 (5/24)	31.2 (44/141)	29.7 (49/165)	n.s.
Lung transplant	[% (*n*/total)]	3.8 (1/26)	0 (0/142)	0.6 (1/168)	**0.019**
Chronic kidney failure	[% (*n*/total)]	20.8 (5/24)	26.4 (37/140)	25.6 (42/164)	n.s.
Heart failure	[% (*n*/total)]	8.3 (2/24)	17.0 (24/141)	15.8 (26/165)	n.s.
Arterial hypertension	[% (*n*/total)]	37.5 (9/24)	57.0 (81/142)	54.2 (90/166)	n.s.
Coronary heart disease	[% (*n*/total)]	8.3 (2/24)	14.9 (21/141)	13.9 (23/165)	n.s.
Diabetes	[% (*n*/total)]	29.2 (7/24)	24.8 (35/141)	25.5 (42/165)	n.s.
Immunosuppression	[% (*n*/total)]	50.0 (12/24)	25.4 (36/142)	28.9 (48/166)	**0.014**
Malignancy	[% (*n*/total)]	29.2 (7/24)	28.2 (40/142)	28.3 (47/166)	n.s.
Solid	[% (*n*/total)]	0 (0/24)	4.9 (7(142)	4.2 (7/166)	n.s.
Haematologic	[% (*n*/total)]	20.8 (5/24)	22.5 (32/142)	22.3 (37/166)	
Solid and haematologic	[% (*n*/total)]	8.3 (2/24)	0.7 (1/142)	1.8 (3/166)	
CCI	[median (IQR)]	3.5 (1.75 – 5.25)	5 (3 – 7)	5 (3 – 6)	n.s.
Clinical presentation and features					
Fever	[% (*n*/total)]	21.0 (4/19)	35.8 (43/120)	33.8 (47/139)	n.s.
Newly reported dyspnea	[% (*n*/total)]	36.8 (7/19)	48.4 (59/122)	46.8 (66/141)	n.s.
URTI	[% (*n*/total)]	33.3 (4/12)	34.0 (33/97)	33.9 (37/109)	n.s.
LRTI	[% (*n*/total)]	63.2 (12/19)	77.8 (84/108)	75.6 (96/127)	n.s.
Bronchitis	[% (*n*/total)]	15.8 (3/19)	10.3 (11/107)	11.1 (14/126)	n.s.
Pneumonia	[% (*n*/total)]	42.1 (8/19)	48.1 (52/108)	47.2 (60/127)	n.s.
Exacerbation of OLD	[% (*n*/total)]	10.5 (2/19)	26.9 (29/108)	24.4 (31/127)	n.s.
ICU stay	[% (*n*/total)]	19.2 (5/26)	21.1 (30/142)	20.8 (35/168)	n.s.
Length of ICU stay [days]	[median (IQR)]	2 (1.5 – 7)	5 (2.75 – 12.5)	4 (2 -10)	n.s.
Ventilatory support	[% (*n*/total)]	11.5 (3/26)	23.2 (33/142)	21.7 (36/166)	n.s.
None*	[% (*n*/total)]	88.5 (23/26)	76.8 (109/142)	79.5 (132/166)	n.s.
HFNC	[% (*n*/total)]	0 (0/26)	1.4 (2/142)	1.2 (2/166)	
Non-invasive	[% (*n*/total)]	3.8 (1/26)	9.2 (13/142)	8.4 (14/166)	
Invasive	[% (*n*/total)]	7.7 (2/26)	12.7 (18/142)	12.0 (20/166)	
Administration of bronchodilators	[% (*n*/total)]	12.5 (3/24)	30.9 (42/136)	29.8 (45/152)	n.s.
Syst. Prednisolone administration	[% (*n*/total)]	12.5 (3/24)	22 (29/132)	20.5 (32/156)	n.s.
Co-infections	[% (*n*/total)]	19.2 (5/26)	27.3 (39/143)	26.0 (44/169)	n.s.
Bacterial	[% (*n*/total)]	7.7 (2/26)	11.2 (16/143)	10.7 (18/169)	n.s.
Viral	[% (*n*/total)]	11.5 (3/26)	10.5 (15/143)	10.7 (18/169)	
Fungal	[% (*n*/total)]	0 (0/26)	0.7 (1/143)	0.6 (1/169)	
Combined	[% (*n*/total)]	0 (0/26)	5.6 (8/143)	4.7 (8/169)	
Mortality	[% (*n*/total)]	3.8 (1/26)	12.0 (17/142)	10.8 (18/166)	n.s.

Analyzed categories are displayed in the column to the left and are either given as frequencies (%) or as median and interquartile range (median (IQR)). (n/total) indicates the respective cases for the total amount of available data. The p-values of the chi-square tests for the contingency tables including all subcategories are indicated. The Mann–Whitney U test was performed to compare continuous variables. CCI, Charlson comorbidity index; HFNC, high-flow nasal cannula; ICU, intensive care unit; LRTI, lower respiratory tract infection; n.s., not significant; OLD, obstructive lung disease; syst., systemic; URTI, upper respiratory tract infection; *including low flow-oxygen via nasal cannula.

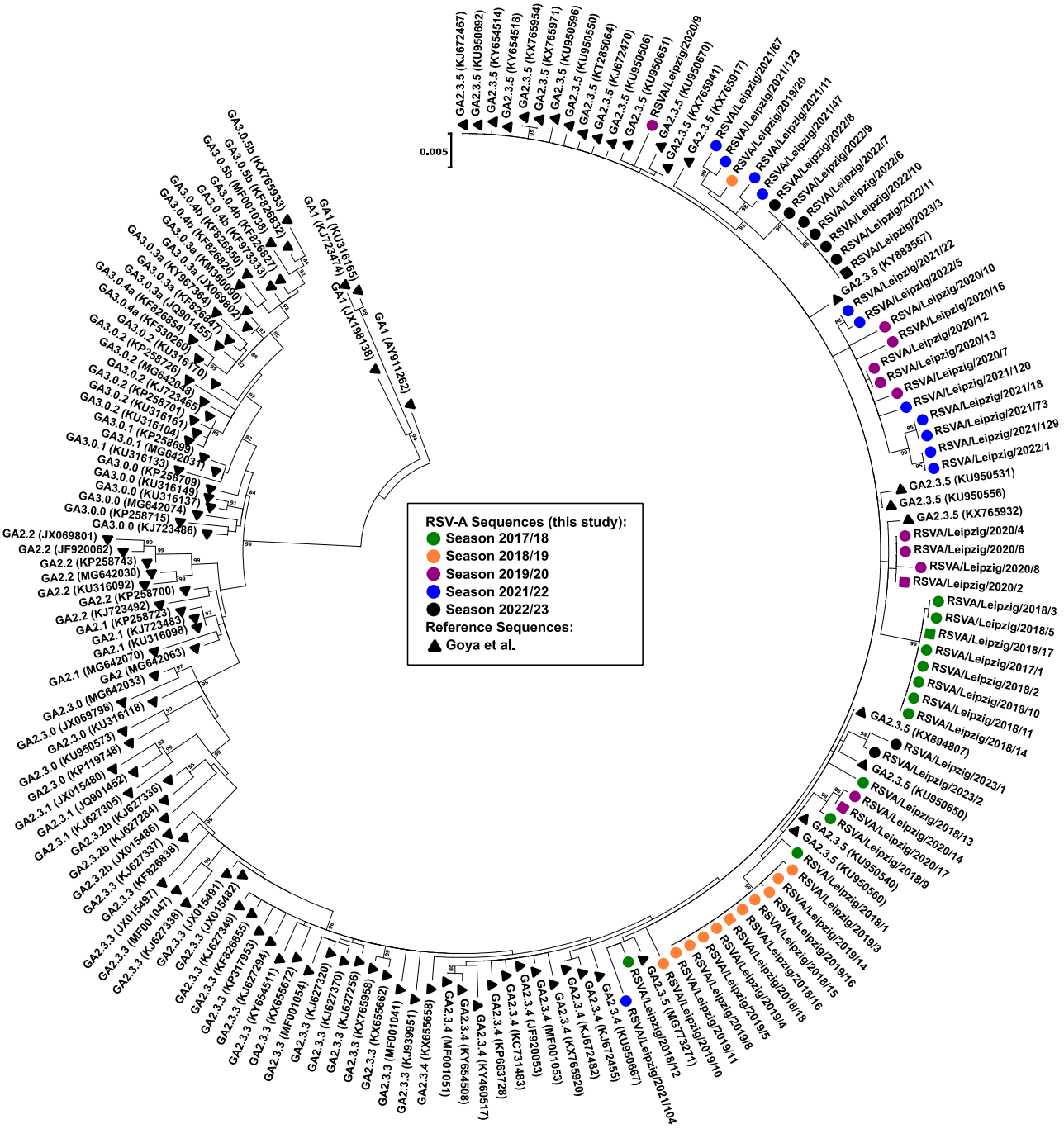

Figure S1. Molecular Phylogenetic analysis of the RSV-A F gene by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [39]. The tree with the highest log likelihood (-8,897.71) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 171 nucleotide sequences. There was a total of 1,725 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [38]. Only nodes with a statistical support >80% are shown. The symbols indicate the sequence origin or the season of the indicated strain: dots/squares: green: season 2017/2018 isolates; orange, season 2018/2019 isolates; purple, season 2019/2020 isolates; blue, season 2021/2022 isolates; black, season 2022/2023 isolates; squares, fatal cases; black triangle: consensus reference sequences according to Goya et al. [16].

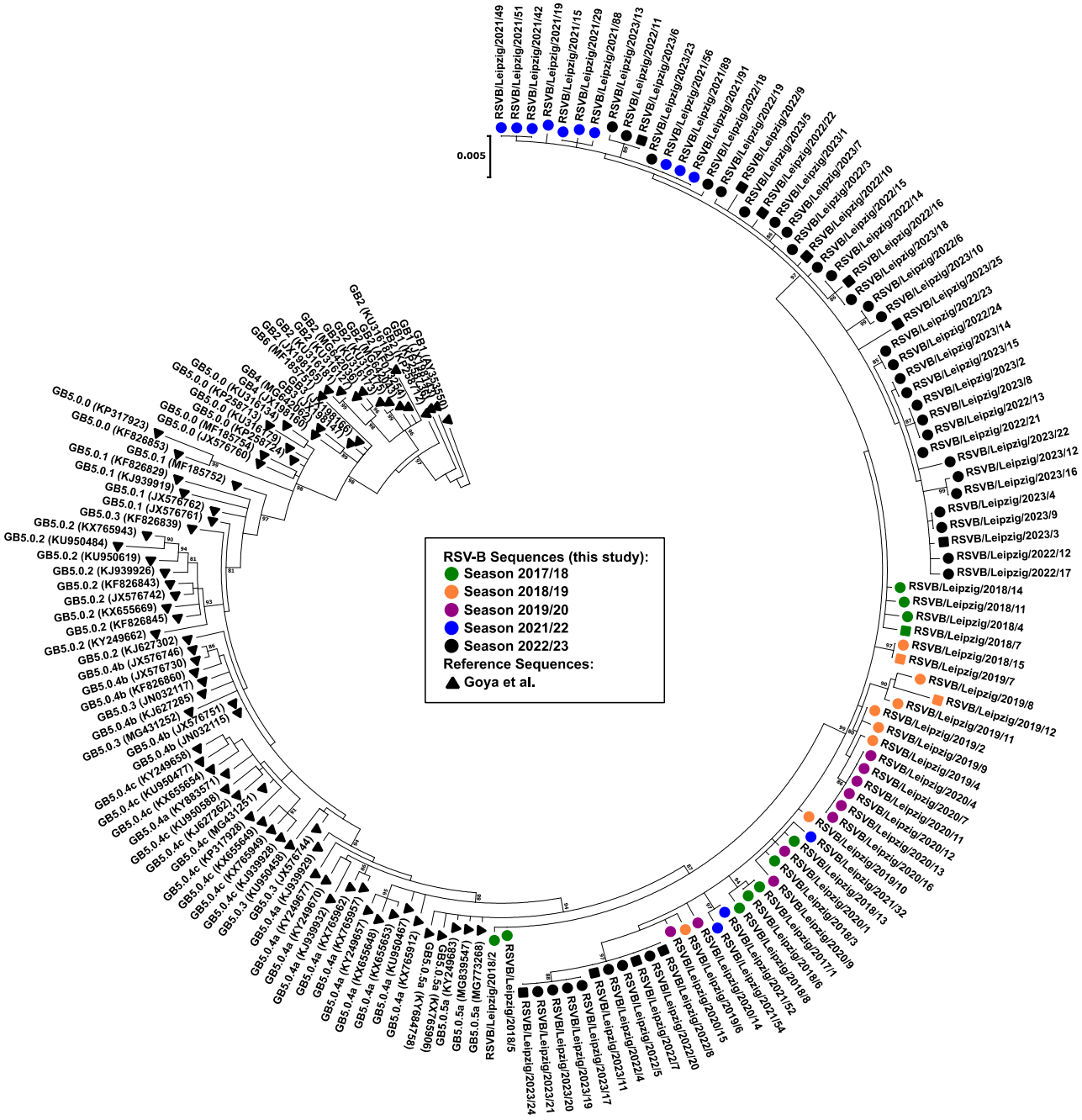

Figure S2. Molecular Phylogenetic analysis of the RSV-B F gene by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [39]. The tree with the highest log likelihood (-7,008.58) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 170 nucleotide sequences. There were a total of 1725 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [38]. Only nodes with a statistical support >80% are shown. The symbols indicate the sequence origin or the season of the indicated strain: dots/squares: green: season 2017/2018 isolates; orange, season 2018/2019 isolates; purple, season 2019/2020 isolates; blue, season 2021/2022 isolates; black, season 2022/2023 isolates; squares, fatal cases; black triangle: consensus reference sequences according to Goya et al. [16].