INTRODUCTION

Viruses in forests and public green spaces

Plant pathogenic viruses are widespread in all ecosystems. They cause significant diseases in a wide variety of host plants and are estimated to be responsible for almost half of all plant diseases occurring worldwide (Anderson et al. 2004; Hilaire et al. 2022). This affects not only the agricultural and horticultural sectors, where enormous global damage is caused by yield and harvest losses due to plant viruses (Sastry and Zitter, 2014; Tatineni and Hein, 2023), but also the forestry sector. However, compared to agriculture and horticulture, studies on viruses in trees of forest and urban ecosystems are still in their infancy.

In many cases, plant viruses cause systemic infections by spreading throughout the plant and infecting different tissues (Waigmann et al. 2004). Viruses can cause diseases manifested by chlorotic discolouration, deformation or necrotic lesions of the leaves. Abnormalities of the flowers or fruits as well as growth disorders also occur. Interactions with the photosynthetic apparatus within the chloroplasts, which are important targets of viruses within infected cells (Bhattacharyya and Chakraborty, 2018), can reduce photosynthetic performances and cause a nutrient deficiency, resulting in a general decline in vigour. Reduced photosynthetic activity affects growth, reproduction and the accumulation of reserves required for stress tolerance. Disturbing the photosynthesis and thus the metabolism of plants can also be achieved by controlling the stomata of plant leaves (Murray et al. 2016).

During virus replication and transport within the plant, the cellular host metabolism is reorganized. This can lead to the degradation of important plant structures. The weakened plant becomes more susceptible to secondary infections by bacteria, fungi or other pathogens. The cumulative combination of viral and secondary infections can result in plant death. Plant viruses can impair the root functions of the plant (Chen et al. 2017). Damaged roots are less able to absorb water and nutrients from the soil, which leads to dehydration and malnutrition. In response to virus infections, plants activate various defence mechanisms such as programmed cell death (Coll et al. 2011). This strategy to limit the spread of the virus can weaken the plant in case of a severe infection.

Examples from horticulture impressively demonstrate the importance of plant viruses for woody plants. Symptoms on various organs can lead to considerable damage and growth depression, resulting in major yield losses. In some cases, trees die and have to be removed from large areas. *Citrus tristeza disease*, caused by the citrus tristeza virus (CTV) from the *Closteroviridae* family, has already wiped out millions of infected trees (Bar-Joseph and Dawson, 2008; Moreno and Garnsey, 2010). CTV infects citrus plants worldwide and has a wide host range that includes many citrus species, including oranges, grapefruits, lemons and limes. In addition to leaf symptoms, disruption of the vascular system and interruption of water and nutrient transport can lead to the death of individual branches and, in severe cases of infection, the entire citrus tree. The loss of infected trees, reduced fruit quality and the need for extensive aphid control measures pose economic challenges for citrus growers (Moreno et al. 2008). Scharka disease, caused by the plum pox virus (PPV) from the *Potyviridae* family, is also a dreaded disease affecting various stone fruit trees of the *Prunus* genus, including plum, peach, apricot and cherry (Barba et al. 2011). The weakening of infected plants, especially in the case of infections with several virus strains, can negatively affect their vitality to such an extent that they die. PPV is mainly transmitted by aphids and can lead to high yield losses in affected growing regions as a result of poor fruit quality and costly control measures (Anonymous, 2023). Cassava mosaic disease in cassava, caused by viruses of the genus *Begomovirus* (Thresh and Cooter, 2005), or diseases in viticulture, which are caused by over 50 known viruses from different families (Andret-Link et al. 2004; Martelli, 2014), are further important examples of the significance of viruses for woody plants.

Even though unknown plant viruses are constantly being identified using methods such as HTS, only a small proportion relates to forest trees. Nienhaus (1985) and Nienhaus and Castello (1989) pointed out that viruses also play a central role in forest ecosystems. In combination with other stressors, viruses are involved in the development of damage symptoms in woody plants. Due to the lack of routine diagnostic methods such as serological ELISA or specific oligonucleotides for molecular detection, comparatively few plant viruses have been detected in forest trees to date, making it difficult to assess their significance (Büttner et al. 2023).

Despite important advances in research on viruses in forest trees in recent decades, studies on fungal pathogens and insect pests in forests are much more common. Studies on ash dieback, which threatens populations of common ash (*Fraxinus excelsior* (*F. excelsior*)) throughout Europe, mostly focus on the fungal pathogen of the disease, *Hymenoscyphus fraxineus* (*H. fraxineus*) (Langer et al. 2022). The extent to which viruses are involved in the pathogenesis is unclear. This can lead to the impression that viral diseases in forest trees are negligible, especially since the symptoms they cause are often difficult to detect by visual scoring and are usually limited to leaf symptoms, which can easily be overlooked due to the large canopy of the trees. However, new technologies in the fields of sequencing and bioinformatics have impressively demonstrated in the emerging field of dendrovirology, that the abundance and diversity of viruses is far greater than previously assumed. Pathogenic plant viruses infect forest and urban trees as well as crops and cause both economic and ecological losses (Büttner et al. 2023). Previously unknown viruses from various families have been identified in a wide variety of deciduous tree species (Rumbou et al. 2021). Investigations into the virome on birch trees suffering from birch leaf-roll disease yielded findings on three previously unknown viruses from the genera *Carlavirus*, *Idaeovirus* and *Capillovirus* (Rumbou et al. 2020).

Damage to plants is usually not of monocausal origin but is based on complex interactions between numerous factors. The concept of the phytobiome encompasses networks of interactions between plants, their environment and the complex communities of organisms associated with them, such as arthropods, fungi, bacteria and nematodes, which greatly influence the health and productivity of plants (Beans, 2017) (Fig. 1). Originally related to agroecosystems, the concept can also be applied to forest ecosystems. One example is the complex disease pine wilt disease. It is the result of an interaction between pine trees, the pine wood nematode *Bursaphelenchus xylophilus* and beetles of the genus *Monochamus*. The nematode is considered a quarantine pest in Europe (Anonymous, 2012). The disease, which is responsible for an enormous decline in conifers in Eurasian coniferous forests, is caused by the endoparasitic nematode. It colonises the above-ground parts of the tree and feeds on the cells surrounding the resin canals. The damage causes resin to penetrate the tracheids, disrupting water transport and causing the tree to wither and eventually die. To colonise new hosts, the nematode uses the beetles, that have just developed in the wood by colonising their respiratory system. When the beetle feeds on the branches of healthy trees, the nematodes leave their vector and enter new host trees through the feeding wounds (Mota and Vieira, 2008).

Being an important part of the plants‘ phytobiome, viruses must also be taken into consideration. They interact with other pathogens, e.g. by using arthropods, nematodes and fungi as biological vectors to be efficiently transmitted from infected to uninfected plants. Viruses of the genus *Nepovirus* are transmitted by various nematodes (Harrison and Murant, 1996). Gall mites are considered to be efficient vectors of viruses of the genus *Emaravirus*, which infect important forest trees such as oak, ash, maple and Eurasian aspen (Rehanek et al. 2022). Viruses can also infect fungi and thereby alter their pathogenicity. Such mycoviruses can lead to an increase (hypervirulence) or decrease (hypovirulence) of the fungal pathogen and are therefore significant in terms of the phytobiome. One example from the forestry sector is white pine blister rust, caused by the fungus *Cronartium ribicola*. The fungus infects various pine trees around the world and caused a 95 % reduction in tree populations in heavily affected stands in western North America (Fins et al. 2002). Studies show that mycoviruses of the *Totoviridae* family may be associated with specific virulent pathotypes of the fungus (Liu et al. 2019). Further studies are evident that recognized the importance of viruses for their plant hosts, for example by improving the host's tolerance to abiotic drought stress (Xu et al. 2008) and thus emphazising their contribution to a robust ecosystem. White clover plants, infected with white clover mosaic virus, were shown to be less attractive to fungal gnats compared to uninfected plants (van Molken et al. 2012). In barley yellow dwarf virus infected wheat, plants had a higher leaf water potential at low water inputs and exceeded uninfected plants regarding growth, seed yields and seed germination (Davis et al 2015). In the concept of mild strain cross-protection, a mild strain of a virus can be used to protect affected plants against infection by a severe viral strain (Pechinger et al. 2019). Although the underlying mechanisms of such findings are rarely understood, it is essential to investigate the influence of viruses on plant and tree fitness in environmental contexts further.

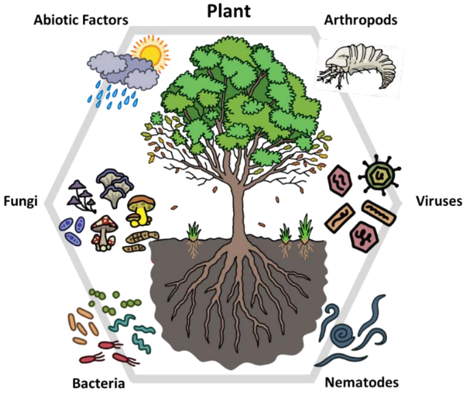

Fig 1. The plants‘ phytobiome. Viruses interact with all biotic components of the phytobiome, including the plants themselves, arthropods, fungi, bacteria and nematodes. Modified after Schoelz and Stewart (2018). Drawings by Hector Colino Fernandez.

Issues relating to the causes of damage to trees will become increasingly important in the future due to climate change and the associated effects (Boyd et al. 2013). Although trees are highly adaptable, the multitude of changes associated with climate change can overwhelm the resilience of trees. An increase in average temperatures and changing precipitation patterns, including more intense heavy rainfall events and longer dry periods, will lead to an increase in droughts and affect water availability for deciduous trees. Photosynthetic performance and growth rates may be reduced by prolonged drought. There may be an impact on the phenological cycle by shifting the timing of leaf emergence and leaf shedding (Vitasse et al. 2011). Extreme events such as forest fires resulting from long dry periods may also become more significant. In connection with a shortage of water availability, the increase in CO_2 concentrations in the atmosphere can influence the metabolic activity of trees. Weakened trees are more susceptible to diseases and pests. The increased occurrence of damaged or dead wood favours further pathogens, which can lead to epidemics. These long-term climate changes will initiate dynamics within forest ecosystems, that are characterized by changing tree species compositions, and an increased risk potential with regard to new diseases (Renner et al. 2021). In such scenarios, viruses need to be considered as contributing factors.

The world of emaraviruses

Emaraviruses have become an important plant pathogenic virus genus in recent years. Rose rosette virus (RRV) and its vector *Phyllocoptes fructiphilus* are listed on the A1 warning list of harmful organisms by the European and Mediterranean Plant Protection Organisation (EPPO) and the UK, and RRV is considered a quarantine pest in Morocco (Anonymous, 2024a). For High plains wheat mosaic virus (HPWMoV), the European Union (EU) plans to classify it as a potential quarantine pest of the Union due to its wide distribution in America, Australia and Ukraine (Bragard et al. 2022). In addition, emaraviruses are significant pathogens for important temperate and boreal forest species in European deciduous trees and public green spaces. Emaraviruses are the only genus within the virus family *Fimoviridae* (order *Bunyavirales*). Within the *Bunyavirales*, they are closely related to tospoviruses and peribunyaviruses. They share similar characteristics with these groups, which include (i) enveloped virions, (ii) a segmented genome, (iii) high sequence similarity of the RNA 1-RNA 3 encoded proteins, (iv) conserved motifs in the amino acid (aa) sequence of RdRP and (v) conserved terminal ends of the individual RNA segments (Elbeaino et al. 2018). Under electron microscopy, emaraviruses can be visualised in the cytoplasm of infected cells as spherical particles with a diameter between 80-100 nm (Mielke-Ehret and Mühlbach, 2012).

European mountain ash ringspot-associated virus (EMARaV) was the first emaravirus described in 2007, as the causal agent of ringspot disease on mountain ash (*Sorbus aucuparia*) (Mielke et al. 2007). In following years, further EMARaV-related viruses were identified in several host plants including fig, rose, maize, pigeonpea and others which led to the establishment of the *Emaravirus* genus (Elbeaino et al. 2018). Currently, the genus comprises 32 species (ICTV, 2024), including viruses that infect important crops worldwide, where they can lead to enormous losses (Kumar et al. 2000; Burrows et al. 2009; Patil et al. 2015; Preising et al. 2021). Ornamental and wild plant species are also infected. With the exception of

HPWMoV and ti ringspot-associated virus (TiRSaV), which infect monocots, most natural host plants are dicots. Emaraviruses are very host-specific and, with a few exceptions, only infect plants of one genus.

Emaraviruses are worldwide distributed, with hotspots in North America, Europe and Asia, although individual species are mainly restricted to one continent. Exceptions to this are HPWMoV and fig mosaic virus (FMV), which have been spread worldwide via infected plant material (Preising et al. 2021; Bragard et al. 2022). While a large number of emaraviruses have been identified in herbaceous plants in North America and Asia, novel emaraviruses have mainly been identified in woody plants in Europe.

Emaraviruses cause a wide variety of symptoms on infected plants. Diseases of deciduous trees that have been observed for a long time, such as shoestring disease of ash and the ringspot disease of oak, can now be related to emaraviruses. The characteristic leaf symptoms were decisive in naming the causal agent (Bandte et al. 2020; Rumbou et al. 2021; Gaskin et al. 2021;). However, emaraviruses can also latently infect their hosts, with the plants remaining asymptomatic during early infection, allowing the virus to spread inconspicuously.

In addition to leaf symptoms, emaravirus infections can also affect fruit quality. For example, deformation, discolouration and necrotisation were observed in fruits infected with jujube yellow mottle-associated virus (JYMaV) (Yang et al. 2019). Similarly, reduced fruit quality was reported in *Rubus* spp. infected with blackberry leaf mottle-associated virus (BLMaV) (Hassan et al. 2019).

Eriophyid gall mites have been described as natural vectors for virus transmission for many emaraviruses (Mielke-Ehret and Mühlbach, 2012). Transmission via grafting or cuttings is common for woody emaraviruses. In some cases, mechanical transmission to herbaceous plants has been experimentally successful.

The emaraviral genome consists of several single-stranded RNA segments, each of which contains an open reading frame. The core genome, consisting of RNAs 1-4, is sufficient for systemic emaravirus infection and the induction of symptoms in *N. benthamiana* (Verchot et al. 2020).

Phylogenetic analyses based on the amino acid sequences of the four core components allow emaraviruses to be divided into four main clades (A, B, C and D) (Rumbou et al. 2021; Kubota et al. 2021).

In addition to the core segments, most emaraviruses contain additional genome segments, most of which code for non-structural proteins with unknown functions. The number of these additional genome segments varies from one for redbud yellow ringspot-associated virus (RYRaV) (Di Bello et al. 2016) to six for perilla mosaic virus (PerMV) (Kubota et al. 2020).

For the routine detection of emaraviruses, reverse transcription polymerase chain reaction (RT-PCR) is established in most cases, using primer sets to detect different genome segments. Serological detection methods, on the other hand, are rarely available as there are no specific antisera for most emaraviruses. This particularly relates to emaraviruses in deciduous trees.

The importance of viruses in forest trees and urban trees is still difficult to assess, as they have so far been given a subordinate role. Despite increased findings of viruses in these environments, there are challenges in our understanding of viruses that infect deciduous trees. There is also a lack of data on financial damage caused by viruses to forest trees. However, characterizing viruses is key to understand their contribution to the health status of their hosts and potential losses they cause to forest and urban trees. Knowledge of their epidemiology and transmission, genetic equipment, correlations with macroscopically visible symptoms and reliable diagnostic methods make it possible to target newly discovered viruses. With this knowledge, complex interactions between viruses and their hosts and the interaction of viruses with other stress factors can be elucidated and sustainable strategies for the management of viral diseases in forests can be developed.

This thesis focuses on two important forest tree species that are affected by emaraviruses, among others, and the characterization of those novel viruses.

Oak is used intensively in forestry, providing important ecosystem services. They significantly contribute to the structure and function of forest ecosystems in their area of distribution and play a crucial role in protecting the soil from erosion and landslides, regulating water flow in water catchment areas and maintaining water quality in streams and rivers. Oak forests also provide habitat and food for numerous species. In California, at least 300 terrestrial vertebrate species, 1,100 native vascular plant species, 370 fungal species and about 5,000 arthropod species are associated with oak forests (Swiecki, 1997). In Europe, the oak is one of the economically and ecologically most important deciduous trees. It provides wood for fuel, bark for tanning, timber for construction and acorns for livestock breeding. At 2.93 %, the common oak (*Quercus robur* L.) is the deciduous tree species with the third highest area share in Europe after the common beech (*Fagus sylvatica*) and the moor birch (*Betula pubescens*) (Hemery, 2008). It is widespread in large parts of Europe, from the north in southern Norway, Sweden and northern Scotland to the south to the Iberian Peninsula, southern Italy, Greece, the Balkans and southern Turkey. In the east it can be found as far as the Urals. Its large ecological amplitude allows it to colonise many locations. It grows on heavier soils in more continental climates, in damp lowlands and humid areas along streams and rivers and also tolerates periodic flooding. It occurs at low to medium altitudes, but also grows in the Alps up to an altitude of 1300 meters and can act as a pioneer tree species. The deep taproots give the common oak stability and enable it to survive moderate periods of drought (Eaton et al. 2016). In Germany, oaks can be found on 10 % of the forested area (Anonymous, 2015).

Diseases of oak trees in forests were first observed in Germany in the middle of the last century by Schmelzer (1963). Subsequent investigations suspected a viral origin as the cause of the symptoms (Nienhaus, 1975). Surveys of oak seedlings and trees in nurseries and forest districts in northern Germany also revealed suspected symptoms such as chlorotic ringspots, which led to a loss of vitality. The symptoms could not be transmitted mechanically in inoculation experiments, but transmission by grafting onto healthy seedlings was successful (Büttner and Führling, 1996).Viruses frequently occurring in forest ecosystems and associated with oaks, such as tobacco mosaic virus (TMV), tobacco necrosis virus (TNV), cherry leaf roll virus (CLRV) and blackberry mosaic virus (BMV) (Nienhaus and Castello, 1989) were excluded by Büttner and Führling (1996). Several years later, CORaV was identified as the causal agent of the ringspot disease in common oak (Bandte et al. 2020).

Among the native ash species, the **common ash** (*F. excelsior*) is particularly important for many forest ecosystems in Europe (Pautasso et al. 2013). It occurs as a mixed tree species in various forest communities (Ellenberg und Leuschner, 2010) and plays an important role in primary and secondary succession (Tapper 1996; Marigo et al. 1996). In urban areas, it is often planted due to its aesthetic value. However, the importance of the manna ash (*Fraxinus ornus*) (*F. ornus*), which is considered to be more climate-adapted, is increasing (Beck et al. 2016). Due to its wood properties, ash is cultivated on a large scale (Fraxigen, 2005; Dobrowolska et al. 2011).

Diseases of ash trees were first described at the same time as those of oaks by Schmelzer (1963) and were later linked to CLRV infection (Hamacher and Quadt, 1991). Gaskin et al. 2021 proved the association of the symptoms with the emaravirus ASaV. After its first description in *F. excelsior*, ASaV was also detected in *F. ornus*. The virus is widespread in Europe, including Germany, Switzerland, Italy, France and Sweden (Svanella-Dumas et al. 2022). Other viruses commonly found in forest ecosystems such as TMV, TNV, CLRV, Arabis mosaic virus (ArMV), tomato ringspot virus (ToRSV) or tobacco ringspot virus (TRSV) have been reported for *F. excelsior* (Nienhaus and Castello, 1989; Büttner et al. 2023). Navarro et al. (2017) referred to a partial sequence of privet leaf blotch-associated virus (PrLBaV) from the *Mayoviridae* family in a *F. excelsior* sample, deposited as raspberry bushy dwarf virus (RBDV) isolate (HM153080) in the NCBI database. Recently, full-length sequences of two closely related species of cytorhabdoviruses from the family *Rhabdoviridae* (Fraxinus gammacytorhabdovirus 1, FraGCRV1 (BK064353.1) and Fraxinus gammacytorhabdovirus 2, FraGCRV2 (BK064354.1)) were compiled from published HTS datasets of *F. excelsior* (Bejerman et al. 2023).

Chapter I of this thesis provides a comprehensive meta-analyses that integrates, connects and evaluates recent findings covering various aspects of emaraviruses. The ‚genome organization‘ chapter represents newly generated data of highly interesting value. Newly identified groups of homologous proteins are presented for the first time. The complex system of accessory proteins and its relevance for the virus taxonomy is discussed. Moreover, chapter I serves as an extensive summary and literature review of the emaravirus group until 2022 and still represents the state of the art on emaravirus research. Since its publication, the number of emaraviruses has further increased. Species identified in grapevine, ash, karaka, anise, mistletoe, mugwort, tree of heaven and kudzu that were already proposed in Rehanek et al. (2022) as putative novel members, have been officially classified (ICTV, 2024), based on genomic, biological, and phylogenetic properties. Meanwhile, another putative emaravirus infecting *Ailanthus altissima* (An et al. 2022) and *Clematis brevicaudata* were reported from China (Yang et al. 2023).

Chapter II and chapter III focus on the emaravirus CORaV in common oak, including investigations on the health status of infected trees, the development of diagnostic RT-PCR-based detection systems and the completion of its genome as a basis for the phylogenetic placement.

Chapter IV gives an overview of viruses affecting common ash which is currently one of the most endangered forest tree species due to ash dieback disease. By a viral survey over three years, the virus diversity was recorded in ashes of different origins and ages. Long-known viruses and novel viruses from different genera were included. The detection of ASaV and its association with specific leaf symptoms is described. Distinct phylogenetic groups of cytorhabdoviruses were detected, that affect common ash, as well as manna ash and red ash. Additionally, signatures of novel viruses from different families have been identified that expand the knowledge about the virome of ash.

Chapter V presents the use of generic primer sets for the emaravirus detection and their application to detect novel emaraviruses in woody hosts. Focusing on the emaraviruses in oak and ash, serological work was done to establish diagnostic ELISAs for the CORaV and ASaV detection. Heterologously expressed native viral nucleocapsid proteins were used for immunization and obtained antibodies were tested for their application in different detection assays. Subsequently, His_6 and GST-tagged CORaV P3, P4 and P5 proteins were used for *in vitro* interaction studies.

Chapter VI finally describes the findings concerning putative vector candidates of CORaV in order to gain more information on the biological transmission of the virus.
Together, the thesis provides valuable knowledge on significant viruses of important deciduous trees enabling future research on this highly dynamic field of forest virology.

CHAPTER I The complex world of emaraviruses – challenges, insights and prospects

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Abstract

Emaravirus (Order *Bunyavirales*; Family *Fimoviridae*) is a genus comprising over 20 emerging plant viruses with a worldwide distribution and economic impact. Emaraviruses infect a variety of host plants and have especially become prevalent in important long-living woody plants. These viruses are enveloped, with a segmented, single-stranded, negative-sense RNA genome and are transmitted by eriophyid mites or mechanical transmission. Emaraviruses have four core genome segments encoding an RNA-dependent RNA polymerase, a glycoprotein precursor, a nucleocapsid protein, and a movement protein. They also have additional genome segments whose number varies widely. We report here that the proteins encoded by these segments form three main homology groups: a homolog of the sadwavirus Glu2 Pro glutamic protease; a protein involved in pathogenicity, which we named "ABC"; and a protein of unknown function, which we named "P55". The distribution of these proteins parallels the emaravirus phylogeny and suggests, with other analyses, that emaraviruses should be split into at least two genera. Reliable diagnosis systems are urgently needed to detect emaraviruses, assess their economic and ecological importance, and take appropriate measures to prevent their spread (such as routine testing, hygiene measures, and control of mite vectors). Additional research needs include understanding the function of emaravirus proteins, breeding resistant plants and clarifying transmission modes.

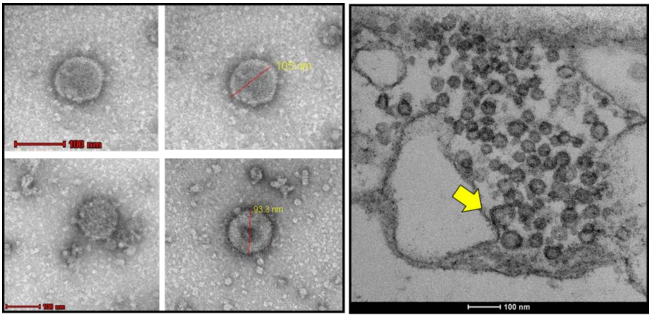
Keywords: diagnosis, distribution, emaraviruses, forest trees, *Fimoviridae*, genome organization, phylogenetic relations, protein domains, symptomatology, transmission

1. Introduction

Emaraviruses are an emerging group of plant-infecting, segmented negative-sense RNA viruses with enveloped particles. They are currently classified as a single genus in the new *Fimoviridae* family (order *Bunyavirales*) [1] and were discovered relatively late by plant virologists. Several virus-like diseases that resisted efforts for long-time to identify the causal agents have now been linked to emaraviruses. For example, viruses causing the rosette disease of rose, the mosaic disease of fig, the sterility mosaic disease of pigeonpea, the ringspot diseases of rowan and oak, or the mosaic disease of aspen trees, eluded the efforts of scientists for decades due to a lack of suitable methods for identification and characterization of such viruses.

Virus-like symptoms such as chlorotic ringspots and mottle on diseased *Sorbus* spp. were first described by Baur [2] and later Kegler [3]. Büttner and Führling [4] reported on the occurrence and distribution of diseased oak trees in Germany, and Ebrahim-Nesbat [5] showed the first electron microscopic images of virus-like particles associated with ringfleck mosaic of mountain ash. A decade later, the identification of a double-stranded RNA pattern and partial sequence data were a cornerstone for the association of the ringspot disease of mountain ash with an unknown virus [6]. The virus, later characterized as European mountain ash ringspot-associated virus (EMARaV) became the first member of a novel virus genus [7,8]. The first description and genetic characterization of EMARaV initiated the identification of similar agents associated with well-known diseases of fig [9], maize [10] and pigeonpea [11,12]. Following the discovery of further related viruses, the International Committee on Taxonomy of Viruses (ICTV) established the newly unassigned genus *Emaravirus* in 2012 with EMARaV, fig mosaic virus (FMV), High Plains wheat mosaic virus (HPWMoV, syn. maize red stripe virus (MRSV), syn. High plains virus (HPV)), pigeonpea sterility mosaic virus (PPSMV), raspberry leaf blotch virus (RLBV), and rose rosette virus (RRV) [8,13]. In 2018, the genus *Emaravirus* was assigned to the novel family *Fimoviridae*, in the *Bunyavirales* order [1]. In the following years, the number of emaraviruses has further increased. Especially in broad-leaved trees, new species of emaraviruses have exclusively been described, including EMARaV in *Sorbus intermedia* [14], *Karpatiosorbus × hybrida* [15], and *Amelanchier* sp. [16], aspen mosaic-associated virus (AsMaV) in *Populus tremula* [17], common oak ringspot-associated virus (CORaV) in *Quercus robur* [18,19], maple mottle-associated virus (MaMaV) in *Acer pseudoplatanus* [20] and ash shoestring-associated virus (ASaV) in *Fraxinus* spp. [21]. Emaraviruses have thereby become the most prevalent group of viruses in long-living plants, causing diseases in key species of the temperate and boreal forests.

To date, 24 species are included in the genus *Emaravirus* (Table 1). Emaraviruses are related to tospoviruses and peribunyaviruses within the *Bunyavirales*, in that they share (i) enveloped virions; (ii) segmented genomes; (iii) high sequence similarity in orthologous proteins for the RNA-dependent RNA polymerase (RdRP, RNA 1), the glycoprotein precursor (GPP, RNA 2) and the nucleocapsid protein (NC, RNA 3); (iv) conserved motifs in RdRP amino acid sequence; and (v) conserved terminal ends of each RNA segment that are nearly complementary to each other [1]. Using electron microscopy, emaraviruses can be visualized in the cytoplasm of infected cells as spherical enveloped particles of 80-100 nm in diameter (Figure 1). Many of them are transmitted by eriophyid gall mites [8].



(a) (b)

Figure 1. Transmission electron microscopic (TEM) visualization of negatively stained emaravirus particles. (a) Spherical particles of approx. 100 nm found in plant homogenates of *Nicotiana tabacum* 10 days after inoculation with ash shoestring associated-virus (ASaV) following negative staining with 2 % ammonium molybdate. (b) Ultrathin section of mesophyll cells of symptomatic, ASaV-infected common ash (*Fraxinus excelsior*) showing double membrane-bound bodies (DMB, yellow arrow) typically found in emaravirus-infected tissues [15]