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PUCCINIALES (Rust fungi): DIVERSITY, HOST INTERACTION, AND EVOLUTIONARY INSIGHTS

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Abstract

Rust fungi are a widespread group of plant diseases that belong to the Basidiomycota division and the Pucciniomycetes class. They are one of the most significant orders of fungi, with about 7000 species. The taxonomy of rust fungi has evolved from early morphological systems to modern phylogenetic classifications, with the current recognition of 11 families and the proposal of a new 18-family system. Rust fungi have complex life cycles that involve five distinct spore stages: spermatia, aeciospores, urediniospores, teliospores, and basidiospores. They can be heteroecious, requiring two unrelated host plants to complete their whole life cycle, or autoecious, completing their entire life cycle on a single host. The review highlights rust fungi's economic and agricultural impact, particularly those that cause diseases on important crops such as Wheat, barley, oats, and sunflower. Rust diseases can cause significant yield losses and continue impacting anthropogenic ecosystems. The ecological significance of rust fungi is also discussed, as they can shape plant community dynamics by selectively affecting specific species or genotypes. Phylogenetic studies of rust fungi are crucial for identifying evolutionary relationships, resolving taxonomic issues, and providing insights into their divergence, speciation events, and evolutionary history. Moreover, these studies provide much-needed information on their divergence, speciation events and evolutionary history. They have shown that there are genetic variations within the group and that there have been increases in gene families that produce secreted proteins, including virulence-effector genes. To sum up, this literature review discusses the significance of studying rust fungi in terms of their complicated life cycles and economic and ecological impacts; it also reflects on what phylogenetic analyses reveal about them. It calls for more studies into the remaining uncertainties concerning classification and evolution among these vast and varied kinds of fungi.

***Keywords*:** Autoecious, evolutionary relationships, economic and ecological, heteroecious, phylogenetic

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Introduction

The Pucciniales order, also known as "Rust Fungi," represents the most complex group of phytopathogens within the Basidiomycota, comprising over 7,800 identified species worldwide (Zhao et al., 2021; Aime et al., 2006). The complex life cycles of these obligate parasitic fungi involve five distinct spore types: basidiospores, spermatia, aeciospores, urediniospores, and teliospores (Zhao et al., 2021). Morphological differences between spores can be used to identify various genera of rust fungi. These differences include the number of cells in the teliospores, the number of germ pores per cell, teliospore germination, the presence or absence of paraphyses in the sorus, the persistence of pedicels, spore ornamentation and staining, and the presence or absence of uredinia (Rezende et al., 2015).

Rust fungi exhibit diverse life cycle patterns: microcyclic, hemicyclic, demicyclic, and macrocyclic, based on the number of spore stages they possess. This variety enhances their complexity and adaptability. As pathogens, some rust fungi species complete their entire life cycle on a single host (autoecious). In contrast, others require two unrelated hosts (heteroecious) to complete their life cycle, adding another layer of complexity (Zhao et al., 2023). The two unique phases of the heteroecious life cycle of rust fungi are the aecial and telial stages/hosts, each of which occurs on a different host. Due to their versatility, many species of rust fungi infect a wide range of agricultural and forest crops, leading to significant economic losses. Moreover, these diseases tend to spread rapidly, posing a severe problem in plant pathology (Zhao et al., 2023; Aime et al., 2018).

The evolution of rust fungi is highly influenced by host specificity. The evolution of infection mechanisms and survival mechanisms have been important in the co-evolution with plants. Rust fungi, for example, may evolve by shifting their host range and adapting to different ecologic niches. Finally, these dynamics are driven by various factors, such as climate change and farming practices (Oberwinkler, 2012). Some case studies help to understand the relationships between several species of rusts, their origins and diversification. The research also used molecular techniques to create phylogenetic trees based on Pucciniales within hosts' families. This has involved putting together sequences from various loci to discover how this group of fungi has evolved over time (Aime et al., 2018). Thus, historical biogeography has also shaped the diversity of rusts through their evolutionary history. Moreover, extant rusts have evolved through several host jumps and shifts among different plant lineages (Aime et al., 2018; McTaggart et al., 2015).

Methodology

Coffee rust samples were collected from Matale and Balangoda districts in Sri Lanka. Morphological analysis was done by observing the collected samples and the spores under the stereo light microscope. Secondary data were collected from authenticated, reputed journals published recently (Google Scholar, Plos One, Pub Med, Research Gate), and data were analysed. A comprehensive literature review was done about the diversity, host interactions and evolutionary insight of order Pucciniales.

Results and Discussion

Taxonomic classification of Rust Fungi (Pucciniales; Basidiomycota)

Rust fungi (Pucciniales) are a widespread group of plant diseases that belong to the Basidiomycota division and the Pucciniomycetes class. They are closely related to Platygloeales, Helicobasidiales, Pachnocybales, and Septobasidiales. With about 7000 species, rust fungi are one of the largest orders of fungi in the fungal kingdom (Aime et al., 2006; Avasthi et al., 2023). Initially, rust fungi were classified into three or four families, Pucciniaceae, Melampsoraceae, and Zaghouaniaceae, based on basidium morphology and teliospore structure (Cunningham, 1931; Sydow & Sydow, 1915). This early classification system is still reflected in the current classification of rust fungi, known as rust fungi

sensu (Aime, 2006), which includes three suborders: Uredinineae, Melampsorineae, and Mikronegeriineae. Cummins and Hiratsuka (2003) proposed a widely used 13-family system that prioritises teliospore and spermogonia characteristics over host associations. However, phylogenetic studies by Aime (2006) and others have shown that several families are polyphyletic or redundant, leading to the current recognition of 11 families.

Because of the genotypic and phenotypic plasticity of rust fungi, the taxonomy of these fungi has been questioned at all levels. However, a recent study by Gautam et al. (2021) and M.C. Aime and A.R. McTaggart (2021) provides a comprehensive taxonomic framework based on 16 years of sampling and molecular data. According to the new classification by Aime and McTaggart (2021), the order Pucciniales is divided into seven suborders and 18 families. These suborders include Araucariomycetineae, Melampsorineae, Mikronegeriineae, Raveneliineae, Rogerpetersoniineae, Skierkineae, and Uredinineae. Additionally, the study recognises 18 new or redefined families, such as Araucariomycetaceae, Crossopsoraceae, Milesinaceae, Rogerpetersoniaceae, and Skierkaceae. The study also introduces four new genera: Araucariomyces, Neoolivea, Rogerpetersonia, and Rossmanomyces. Furthermore, Aime and McTaggart (2021) propose 21 new combinations and one new name. Another important finding is the division of the suborder Melampsorineae into 16 genera based on a phylogenetic framework constructed using rDNA sequences of 160 species. In fact, Zhao et al. (2022) established a new genus called Nothopucciniastrum by segregating it from the polyphyletic Pucciniastrum and proposed ten new combinations.

According to Aime and McTaggart (2021), the higher-rank classification of the Pucciniales order considers morphology, host range, and life cycle characteristics. Recent studies have emphasised the importance of using morphologies of spore-producing structures (basidia, spermogonia, aecia, uredinia, telia) for higher-rank taxonomy. In contrast, spore morphologies (basidiospores, spermatia, aeciospores, urediniospores, and teliospores) are more suitable for generic and species-level taxonomy (Zhao et al., 2022). Teliospores play a crucial role in taxonomy, as their morphology and ontogeny are used to define spore states. Host specialisation has also led to the recognition of formae speciales in some species (Gautam et al., 2021). Differentiating between *Puccinia* species relies on features such as urediniospore size and surface echinulation, germ pore number, shape of uredinial paraphyses, and teliospore hilum width (Liu & Hambleton, 2010). The taxonomy of rust fungi has evolved from early morphological systems to modern phylogenetic classifications. The new 18-family system provides a robust framework for this large and complex group while highlighting the need for further sampling and molecular data to resolve remaining uncertainties.

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General characteristics and life cycle

Most rust fungi have complex and variable life cycles that involve five distinct spore stages: spermatia (pycnia), aeciospores, urediniospores, teliospores, and basidiospores. Each stage plays a crucial role in the life cycle (Newcombe, in Encyclopedia of Forest Sciences, 2004). Rust fungi, such as Black Stem Rust (*Puccinia graminis*), possess all five spore types and require two unrelated host plants to complete their entire life cycle.

This heteroecious life cycle typically starts with the overwintering teliospores, which germinate in the spring to produce basidiospores. These basidiospores infect the primary host, often a different plant species, and initiate the aecial stage, where spermatia and aeciospores are formed. The aeciospores then infect the secondary host, known as the telial host, where the uredinial stage begins, producing urediniospores that can quickly spread the infection. As the growing season progresses, the fungus transitions to the telial stage, producing thick-walled teliospores that can endure harsh winter conditions and restart the cycle (Aime et al., 2018).

In some cases, rust fungi that complete their entire life cycle on a single host are sometimes called autoecious. Microcyclic rusts, which lack the uredinial and aecial stages and the spermogonial stage, are all autoecious. Macro- and demi cyclic rusts, which have all five stages but no initial stage, can be autoecious or heteroecious. *P. allii* is a macrocyclic (fully cycled), autoecious rust, usually producing pycnia, aecia, uredinia, telia, and basidia (promycelia) on a single host (Anikster et al.,2004).

According to previous studies, the shortest life cycles (macrocyclic) with three or fewer spore stages are more evolutionarily advanced forms than the complex life cycles (macrocyclic). However, the overall complexity of rust fungi life cycles and their host specificity remain areas of active research, with implications for understanding the coevolutionary dynamics between these parasitic fungi and their plant hosts (Anikster et al.,2004).

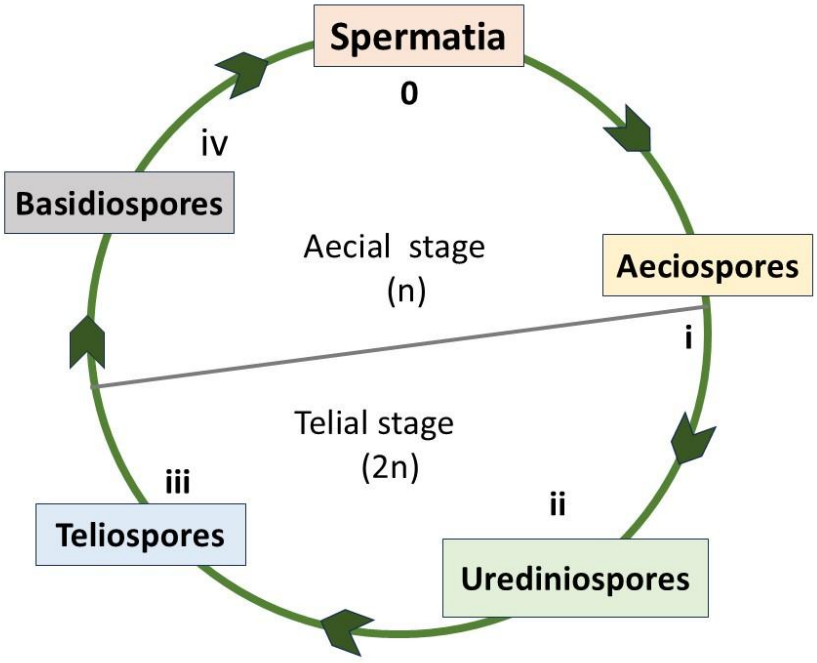

Fig 1: Typical life cycle of rust fungi as in *Puccinia graminis.* Basidiospores (iv) can infect the specific primary host (aecial host) for prophase. Monokaryotic spermatia (0) develop and fertilise

aeciospores (i) at the initial stage on the same host. Dikaryotic aeciospores are then distributed randomly to reach their secondary host (telial host). On that one, the vegetative propagules, urediospore (ii), develop in high amounts for effective distribution. Finally, the teliospores (iii) develop on the same host.

Impact of Rust Fungi

Economic and agricultural impact

Rust fungi, specifically those in the order Pucciniales, are known to cause diseases in important crops. These fungi have continued impacting anthropogenic ecosystems, leading to epidemics and localised extinctions of host plants (Carefoot & Sprott, 1967; Ordonez et al., 2009; Carnegie & Pegg, 2018). Rust diseases have become a significant threat to food production and ecosystems worldwide. Among the various rust fungi species, Puccinia species that cause rusts on cereals are particularly economically important. These species have a heteroecious life cycle, wherein the asexual (uredinial) phase infects cereal hosts, while the sexual phase occurs on different host plants. For instance, *Puccinia coronata* primarily infects oats (Ying Ho et al., 2021); *P. graminis* infects Wheat, barley, and oats (Williams, 1984); *P. helianthi* infects sunflower; *P. hordei* infects barley, *P. purpurea* infects sorghum, *P. melanocephala* infects sugarcane, *P. recondita* mainly infects rye, *P. sorghi* infects maise, *P. striiformis* mainly infects Wheat and barley, *P. triticina* infects Wheat, and *P. malvacearum* infects hollyhock. These rust fungi, specifically *P. graminis, P. triticina*, and *P. striiformis* are responsible for severe seasonal diseases in India and have caused serious outbreaks in North America, Mexico, and South America.

Rust diseases can cause significant yield losses in Wheat and other cereal crops, leading to severe negative economic impacts. For instance, it has been estimated that the average annual global losses due to stripe rust (*P. striiformis* f. sp. *tritici*) and stem rust (*Puccinia* et al.*)* on Wheat were $979 million and $1.12 billion, respectively (Beddow et al., 2015; Pardey et al., 2013). In Australia alone, the cost of controlling these diseases through fungicides is estimated to be $40-90 million annually. Even though wheat leaf rust does not typically cause severe damage if the infection occurs early, it can still yield losses exceeding 50% of disease-free yields in specific locations and years (Huerta-Espino et al., 2011).

Ecological significance

Rust fungi can infect various types of plants, including trees, shrubs, grasses, and crops. They can target different plant parts, such as leaves, stems, roots, fruits, and other areas. However, extensive research has focused on host specialisation among rust fungi. For instance, *Puccini graminis* (stem rust) collected from infected wheat plants show only slight pathogenicity to oat and rye plants. On the other hand, *P. graminis* collected from oats and rye exhibit weak pathogenicity to Wheat (Ordonez et al., 2009). As a result, rust diseases shape plant community dynamics by selectively affecting specific species or genotypes. This significant factor can influence the relative abundance of different plant species, potentially altering the structure and composition of the ecosystem (Liu et al., 2022; Ordonez et al., 2009).

Interactions between rust fungi and plants have clear negative consequences for the hosts. These effects range from the death of seedlings, saplings, and even mature trees to reduced growth throughout all age classes (Avasthi et al., 2023; Liu et al., 2022). Severe infections of rust fungi can result in cankers, galls, premature defoliation, broken limbs and tops, decreased photosynthetic ability, and stunted growth, ultimately leading to decreased yields or even plant mortality. As a result, natural ecosystems, forestry, and agricultural productivity may all suffer.

Certain rust fungi can better adapt to temperature, precipitation, and air composition variations than others. For example, due to its superior environmental adaptation, wheat leaf rust (caused by *P. triticina*) is more widely spread than either stem or stripe rust (Huerta-Espino et al., 2011; Kolmer et al., 2009). Rust diseases have the potential to spread more extensively or adapt to new environments, posing novel threats to ecosystems and plants as a result of climate change.

Environmental factors play a crucial role in the distribution of rust fungi, regardless of geographical distances. Urediniospores, produced at the uridinia stage and economically significant, are carried by wind currents and deposited on the host surface, often facilitated by rainfall events (Liu et al., 2022). Rust diseases in Wheat (*Triticum aestivum*) are of global importance, and uredospores can be dispersed by wind over long distances, without regard for national or geographical boundaries (Liu et al., 2022; Xiu et al., 2023).

Evolutionary Relationships within Pucciniales

According to early hypotheses, the evolution of rust fungi primarily occurred through co-evolution with host plants, starting with ferns and progressing to gymnosperms and angiosperms (Savile, 1971). However, subsequent phylogenetic research challenged this hypothesis and provided a better understanding of the evolutionary relationships within the group (Hart, 1988; Sjamsuridzal et al., 1999). Some studies do support the hypothesis that the aecial stage of rust fungi shows more substantial evidence of co-evolution with hosts than the telial stage by suggesting that co-evolution may play a significant role in shaping the relationships between rust fungi and their aecial hosts (Aime et al., 2018). Additionally, the wheat stem rust fungus *Puccinia graminis* has different forms (forma specialis) that are specialised to infect specific cereal crops such as Wheat, oats, and rye (Anikster, 1984). According to Ordonez et al. (2009), this specialisation results from the coevolutionary process between the rust fungus and its host plants. Early attempts at molecular studies have provided evidence for divergence through co-evolution or from taxonomically small host shifts in rust fungi, such as genera in Phragmidiaceae and Raveneliaceae on species of Rosaceae and Fabaceae (Aime, 2006), as well as species of Endoraecium and Uromycladium on Acacia in Australia (McTaggart et al., 2015). Recent studies suggest that large host jumps can drive the evolution of the rust fungal lineage, indicating that rust fungi can adapt to and infect a wide range of host plants, leading to their diversification (McTaggart et al. et al., 2016; McTaggart et al., et al., 2016). Host jumps have been crucial in diversifying rust fungi, allowing them to adapt to new hosts and become widespread pathogens on various plants in less than 160 million years (McTaggart et al., 2015). According to known molecular data, the most recent common ancestor of modern rust fungi may have evolved on hosts in early gymnosperm lineages like Taxales and Araucariaceae. McTaggart et al. (2015) state that their research supports the hypothesis that rust fungi are relatively new, having a common ancestor on a flowering plant.

Additionally, the study revealed that rust fungi have a most recent common ancestor that is, on average, between 113 and 115 million years old, dating them to a time earlier than previously believed—the Cretaceous period. These studies on the ancestral hosts of rust fungi offer insights into their evolutionary history and their adaptation to different plant lineages (Aime, 2006). According to Silva et al. (2015), the expansion of lineage-specific gene families, the loss of specific genes, and the reduction in carbohydrate-active enzymes are distinctive features in the evolutionary history of rust fungi.

Genetic Markers in Evolutionary Studies

Genetic markers are essential in studying rust fungi evolution as they provide valuable insights into their phylogenetic relationships, genetic diversity, and evolutionary history. A significant breakthrough in evolutionary research on rust fungi was the development of rust-specific primers for three regions of

ribosomal DNA (rDNA): the internal transcribed spacer (ITS), large subunit (LSU/28S), and small subunit (SSU/18S). Previous evolutionary studies on rust fungi have primarily focused on the LSU and SSU regions, particularly at the infrageneric and infrafamilial levels (Aime, 2006; Beenken, 2017; Maier et al., 2003; Scholler & Aime, 2006; Yun, Aime et al., 2011).

The second-largest subunit of RNA polymerase II (RPB2), β-tubulin (B-tub), and translation elongation factor 1 alpha (TEF) have all been effectively used at the species level to determine relationships within specific genera of Pucciniaceae. This family is the most prominent family of rust fungi (Liu & Hambleton, 2010, 2013). The cytochrome c oxidase subunit 3 (CO3) gene of mitochondrial DNA was identified as a barcode for rust fungi (Vialle et al., 2009) and has subsequently been used in most of the phylogenetic studies related to rust fungi (Beenken, 2014; Doungsa-ard et al., 2015; Feau et al., 2011; McTaggart et al., 2015; McTaggart et al., et al., 2016; McTaggart et al., et al., 2016). Previous studies have heavily relied on the ITS region in combination with the intergenic spacer or LSU regions of rDNA to determine relationships between closely related species of rust fungi (Beenken, 2014; Beenken & Wood, 2015; Beenken et al., 2012; Demers et al., 2017; McTaggart et al., 2014; McTaggart et al., et al., 2016; McTaggart et al., et al., 2016). However, according to Alaei et al., 2009 and Virtudazo, Nakamura, & Kakishima, 2001, the ITS region is not a reliable marker for molecular barcoding and direct Sanger sequencing in Puccinales. This is due to observed intraspecific and intra-isolate diversity within a wide range of rust fungi species.

Mechanisms of host adaptation

The biological level of forma specialis (f. sp.) refers to the separation of rusts based on host association within a single species. Leaf rust of Wheat was found to be sexually incompatible with members of the same genus *Puccinia recondita* that had alternate hosts in the Boraginaceae. This led to considering leaf rust on Wheat as a separate species (*Puccinia triticina* Eriks.) (Ordone et al., 2009; Xia et al., 2018). Therefore, many other plant pathogens, including rust fungi, use mechanisms to establish a specific plant-pathogen relationship. In most cases, plant pathogens produce effector proteins that can manipulate host cellular processes and suppress immune responses, enabling them to establish infections in new hosts (Thines, 2019). A study on genomic features related to the obligate biotrophic lifestyle of rust fungi, which includes expanded lineage-specific gene families, showed that rust fungi produce large amounts of effector-like small secreted proteins (Duplessis et al., 2011).

Furthermore, genome analysis of rust fungi revealed that most of their transcripts are differentially expressed in infected leaves. This includes up-regulated transcripts coding for secreted peptidases, lipases, transporters, and carbohydrate-cleaving enzymes essential for host infection and nutrient acquisition (Duplessis et al., 2011). Pathogens can change their genetic composition through genetic mutations or recombination events, leading to adaptations to new host environments. These adaptations may include changes in virulence factors or host recognition molecules (Xia et al., 2018).

A study on the wheat and barley stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) provides genomic insights into host adaptation. The study suggests that gene loss plays a significant role in the evolution of host adaptation in filamentous plant pathogens, especially at the specialist level (Xia et al., 2018).

In the study, specific genes were present in one sample but not the other, indicating the loss of homologs. This loss may be caused by transposable element activities or losses of genomic segments. For example, the evolution of host adaptation in *P. striiformis* is driven by massive transposable element activities, similar to other fungi (Xia et al., 2018).

The high genomic variation within dikaryotic urediniospores of *P. striiformis* has contributed to genome evolution. This variation has resulted in extensive gene losses and occasional gene gains during the divergence of formae speciales (Xia et al., 2018).

Pathogens can gradually expand their host range by acquiring genetic variations that enhance their ability to infect new hosts. Studies have shown that most plant pathogens exhibit differences in patterns and numbers of telomeric repeats between isolates, suggesting that changes in telomeric regions may contribute to host adaptation (Xia et al., 2018). In some instances, plant pathogens can also alter their physiological structures to adapt to the extreme conditions and defences of new host species, enabling them to evade host immune responses (Thines, 2019). Rust pathogens, for example, may co-evolve with their hosts over time, developing adaptations that allow them to overcome host defences and establish successful infections (Thines, 2019). Furthermore, the interaction between pathogens and other pathogens in the host environment can play a role in acquiring traits that enhance their ability to invade new hosts. Many plant pathogens can interact with the host microbiome, influencing their adaptation to new hosts by manipulating host immune responses or nutrient availability (Thines, 2019). Rust fungi secrete large amounts of effector-like proteins that are likely involved in manipulating the host immune system and facilitating parasitic colonisation (Duplessis et al., 2011). Collectively, these mechanisms contribute to the pathogens' ability to adapt to new host species and environments.

Examples of host shifts and co-evolution

Host shifting, also known as host jumping or switching, refers to a pathogen species transitioning from one host species to another, which may be phylogenetically distant from its original host. This evolutionary event is crucial for the successful colonisation and adaptation to a new host species. Host jumping involves mechanisms for pathogens to survive and diversify over evolutionary history. It can lead to pathogen speciation and the development of new interactions and relationships between pathogens and their hosts. This process is influenced by factors such as effector proteins, genetic changes, physiological adjustments, co-evolution with hosts, interactions with other pathogens, and host-microbiome interactions (Sharma et al., 2014; Vagi et al., 2007; Telle et al., 2011; Cooper et al., 2002).

The study suggests that host jumps, whether between taxonomically immense hosts or within the same family, were the main drivers of the diversification of rust genera (McTaggart et al., 2015). This contrasts strict co-evolution with hosts (McTaggart et al., 2015). On the other hand, Rust fungi typically jump hosts during their life cycles, moving between several host species or even host kingdoms at different times of their growth. By forming separate lineages within the fungal group, these host jumps can result in genetic differences and adaptation to new host conditions, which are important for the diversity and speciation of rust fungi (Merwe et al., 2004; Aime et al., 2018). According to McTaggart et al. (2015), rust fungi have become widespread pathogens on various plants in less than 160 million years due to host jumps.

Previous studies have observed host shifts in species within the genus *Phakopsora* (Maier et al., 2015), genus *Puccinia* (van der Merwe et al., 2008; McTaggart et al., 2014), *Uromycladium* (Doungsa-ard et al., 2015), and within genera of the Mikronegeriaceae and Sphaerophragmiacea families. These studies indicate that some rust fungi can shift to new host plants, allowing them to adapt and infect a broader range of hosts. Therefore, these findings support the idea that hosts and their parasites do not always result from long-term co-evolution.

However, the *Endoraecium* species that infect Acacia demonstrate a case of co-evolution. This indicates a close and specific evolutionary relationship between these rust fungi and their host plant, Acacia.

Additionally, the study also shows examples of co-evolution within genera of the Phragmidiaceae family on hosts in the Rosaceae family, suggesting a long-term evolutionary association between these rust fungi and their specific host plants (McTaggart et al., 2015).

Examples of Rust fungi

Rust fungi on Coffee

Hemileia vastatrix is the causal agent of Coffee Leaf Rust (CLR) and belongs to the Phylum Basidiomycota, Class: Pucciniomycetes, Order: Pucciniales, Family: Pucciniaceae, and Genus: *Hemileia* (Valdiviezo et al., 2024). In terms of host range, *H. vastatrix* primarily affects domestic and wild *Coffea* spp., including the majority of *C. arabica* varieties and some *C. canephora* varieties as well (Gullino ML, 2021; Valdiviezo et al., 2024).

Due to the diverse disaster caused by the discovery of CLR, *H. vastatrix* is categorised as primitive rust. The first recorded appearance of *H. vastatrix* occurred in the 1869 epidemic in Sri Lanka (Talhinhas et al., 2014; Talhinhas, 2019; Talhinhas, 2021). A biotrophic fungus requires a living host to survive, obtain nutrients, and complete its reproductive cycle. Compared to *H. coffeicola*, another rust fungus that can infect *C. arabica*, only *H. vastatrix* economically affects coffee-growing countries globally (Talhinhas *et al*., 2014).

Hemileia vastatrix exhibits distinct morphological features, including the shape and structure of its spores (uredinospores and teliospores), the formation of sori on host plant tissues, and other reproductive structures. The uredinospores of *H. vastatrix* are ovoid/reniform single-celled structures with thick walls and hydrophobic surfaces. They have protuberances/spines on the dorsal and convex faces and smooth surfaces on the ventral and concave faces (Valdiviezo *et al*., 2024). On average, mature uredinospores can grow up to 300 spines, which function to grip and hold on to foliar tissues (Voegele *et al*., 2009). *H. vastatrix* teliospores have a smooth surface, and both uredinospores and teliospores have hyaline walls with a thickness of 1 µm. The formation of supra-stomatal sori with bouquet shapes is another characteristic feature of *H. vastatrix* (Talhinhas et al., 2014).

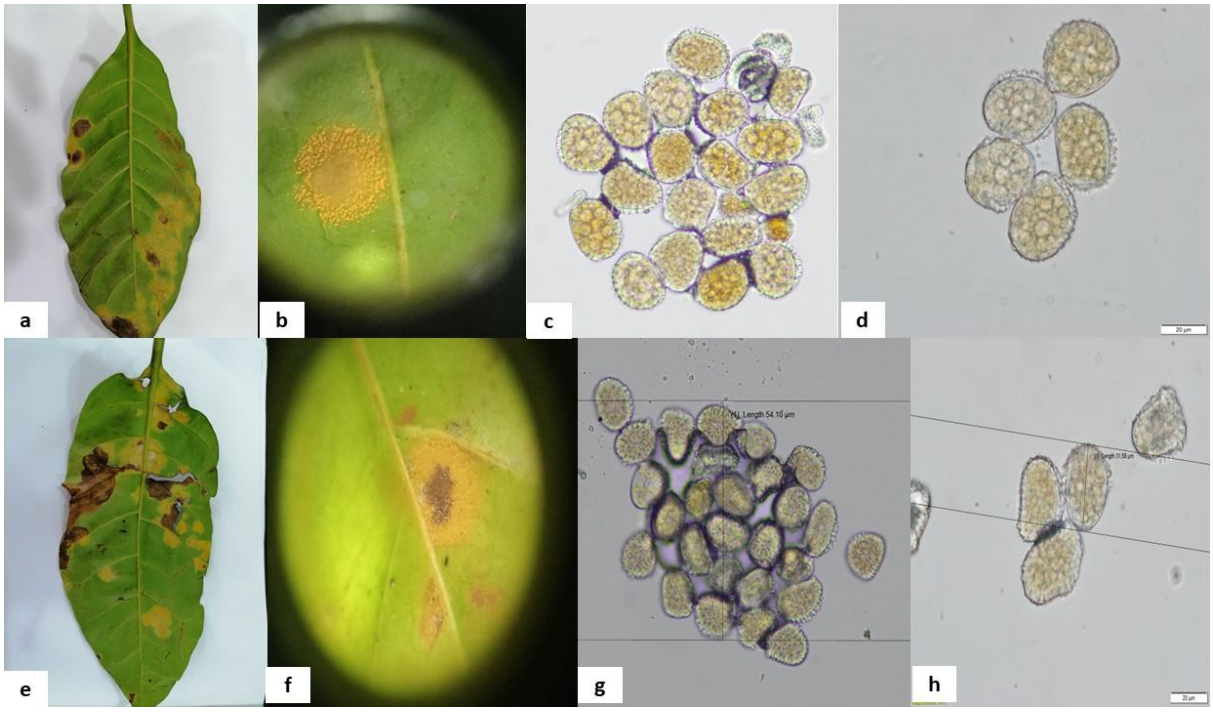

Fig 2: Collected coffee rust samples from Matale and Balangoda in Sri Lanka. (a) Surface of the rust-infected coffee leaf sample (b) Stereomicroscopic view of rust fungi (c) Microscopic view of rust fungi pustule (d) Microscopic view of rust fungi spores from Matale. (e) Surface of rust-infected coffee leaf sample (f) Stereomicroscopic view of rust fungi (g) Microscopic view of rust fungi postulates (h) Microscopic view of rust fungi spores from Balangoda.

Rust fungi on Wheat

Puccinia triticina causes wheat leaf rust, also known as brown rust. It is the most common fungal disease in Wheat and could lead to significant yield loss of 20%. This disease is devastating in temperate regions where the pathogen can survive through the winter cropping season and infect winter crops. The infected plants have small, yellow spots on the upper leaf surface, which change into orange-coloured pustules. On such leaves, pustules appear to release spores, resulting in a scenario that looks like an "orange dust" on both the leaves and surrounding areas. *Puccinia triticina* has a complex life cycle comprising both sexual and asexual reproduction. It forms five types of spores: teliospores, basidiospores (on Wheat), urediniospores, and pycniospores plus aeciospores (on alternate hosts). *Puccinia triticina's* primary host includes common Wheat (Triticum aestivum) durum wheat among several other species of Wheat, while Thalictrum speciosissimum, which belongs to Ranunculaceae family, serves as its alternate host (Bolton et al., 2008; Huerta-Espino et al., 2011).

The common name for stripe rust, caused by Puccinia striiformis, is yellow rust. The major symptoms of the disease are yellow-orange stripes on the leaves, which can cause significant yield losses in Wheat, especially in cooler regions. The disease has been described as elongated yellow-striped lesions and leaf margins that might become tan or brown with age. In addition, like leaf rust, spores of stripe rust need moisture to germinate and proliferate within short periods. *Puccinia graminis* causes stem rust, also known as black rust. It mainly affects stems but can also attack leaves and grains. Initial symptoms comprise oval to elongate reddish-brown lesions that will eventually develop into black pustules during advanced stages of infection. Stem rust follows a life cycle similar to leaf and stripe rusts, requiring both wheat crops and an alternate host for sexual reproduction (Hovmøller et al., 2011; Jin et al., 2010).

Puccinia graminis is a pathogenic fungus that causes black stem rust, a significant disease affecting various grasses, particularly cereal crops like Wheat and barley. It belongs to the order Uredinales and is heteroecious, requiring two different host plants to complete its life cycle. Aecial hosts are usually berberidaceous, whereas telial hosts are predominantly poaceae (grasses). The life cycle of *P. graminis* consists of several spore stages as follows: urediniospores, teliospores and basidiospores. Also, this fungus can produce massive amounts of urediniospores, which, during the growing season, spread the disease (Abbasi et al., 2005; Olivera et al., 2017).

Conclusions and Recommendations

Rust fungi, belonging to the order Pucciniales, are a diverse group of plant pathogens with significant economic and ecological impacts. This review article overviews their diversity, host interactions, and evolutionary insights. The evolution of the Pucciniales order remains a complex and multifaceted problem in plant pathology and evolutionary biology. Despite considerable advances in recent years, several evolutionary questions persist regarding the evolution of Pucciniales and the adaptations that enabled them to become the largest and most complex group of plant pathogens. For example, the complex heteroecious life cycle of rust fungi has led to several hypotheses about how the co-evolution between the fungi and their hosts has driven the diversification of the Pucciniales order. However, no studies have directly tested these hypotheses about the coevolutionary processes shaping the diversity of rust fungi. The article highlights the need for further research to address the complexities of rust

fungi life cycles, their significant impacts, and the evolutionary insights that can be gained through advanced phylogenetic studies.

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