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## Coprophilous fungi in the search for new antimicrobials and other beneficial natural products†

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Microbial interactions involve complex processes shaped by their ecological contexts. Herbivore animal dung denotes an interesting ecological niche for the study of interorganism communication and competition mediated by small molecules. Coprophilous organisms, which inhabit or are associated with animal dung, have developed resourceful defense mechanisms to survive in this competitive environment. Fungi, in particular, are renowned for their ability to produce biologically active secondary metabolites, a chemical arsenal that fosters successful colonization of the dung substrate. With recent advancements in OMICs technologies and our extensive knowledge of coprophilous fungi diversity, we can now delve into the biosynthetic machinery of these organisms and explore the opportunities they offer for discovering new antimicrobials and other beneficial natural products. This review explores the potential of coprophilous fungi in the context of the intricate microbial dynamics of this substrate, particularly the biosynthetic and chemical diversity that make this environment especially promising for natural product discovery. Notably, taxa spanning multiple families within the Sordariomycetes, Dothideomycetes, and Eurotiomycetes have been reported to thrive in dung, highlighting their potential as a reservoir of unique metabolic capabilities. Indeed, 198 secondary metabolites, derived from polyketide, amino acid derived, terpene, and hybrid pathways, have been reported from these fungi, underscoring the remarkable scope of their biosynthetic potential.

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### 1. Introduction

Fungi are widespread across diverse ecosystems, playing a key role in their maintenance and intrinsic dynamics.<sup>1,2</sup> In fact, fungal diversity results in part as a consequence of the ecological pressure and continuous adaptation required for survival. These fascinating organisms have thus developed various mechanisms to interact with their environment and with other organisms within different ecological niches.<sup>3</sup>

Coprophilous fungi, for instance, represent a unique example of these adaptive mechanisms. These taxa, which live or are associated mostly with the dung from herbivores, have developed creative strategies to survive in challenging conditions.<sup>4</sup> One of the several traits that facilitates the survival of coprophilous species is their ability to produce a vast array of biologically active secondary metabolites. The process of dung

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colonization is a complex phenomenon, and our understanding on interspecies interactions remains limited. However, as in the case of *Podospora araneosa* (syn. *Sordaria araneosa*), it is well known that the production of potent antifungals, such as the sordarins, supports the successful colonization of this fungus by inhibiting the growth of rapid opportunistic species.<sup>5,6</sup> Similarly, *Stilbella fimetaria* (syn. *S. erythrocephala*) produces antiamoebic peptaibol antibiotics, which are secreted at inhibitory concentrations in rabbit dung pellets, allowing this fungus to flourish in the presence of bacterial and fungal competitors.<sup>7</sup> Despite the limited tools available to study these type of interactions in nature, coprophilous fungi have been harnessed over the years as an important source of new antimicrobials and other potentially beneficial natural products.

The present review aims to provide updated information on the chemical diversity of the secondary metabolites (SMs) produced by coprophilous fungi, their biosynthetic origin, and their biological properties. In addition, the different implications of their SMs are discussed in light of the advances in natural product chemistry and current OMICs techniques, as in the case of genomics and metabolomics. Our goal is to establish a solid framework for the future study of the biosynthetic potential of coprophilous fungi, leading to the discovery of promising natural products with interesting biological properties that can serve as leads to fuel development of novel therapeutics. Furthermore, we emphasize the need to address existing knowledge gaps that span technical methodologies, infrastructure, and other critical aspects of the field.



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## 2. Coprophilous fungi: diversity and isolation

Coprophilous fungi are those that inhabit or are associated with animal dung. Herbivorous animal dung provides a nutrient-rich substrate for fungal growth, as it contains cellulose, hemicellulose, lignin, minerals, and high levels of nitrogen and moisture.<sup>8</sup> The trophic capabilities of some dung-inhabiting fungi, such as *Preussia* and *Sordaria*, reveal adaptations to this specific substrate. In contrast, other more ubiquitous taxa, including *Chaetomium* and *Penicillium*, likely colonize dung opportunistically through generalist strategies rather than specialized ecological or metabolic adaptations. Among coprophilous fungi (Fig. 1), representatives of Ascomycota, Basidiobolomycota, Basidiomycota, Mucoromycota, and Zoopagomycota can be found, the latter being the most common division.<sup>9,10</sup> Advances in fungal systematics, particularly through multilocus genomic sequence analyses of ribosomal DNA (18S rDNA, ITS1/2, 5.8S rDNA, 28S rDNA) and protein-coding genes (*e.g.*,  $\beta$ -tubulin, translation elongation factor 1 $\alpha$ , and RNA polymerase II subunit RPB2), have significantly enhanced the resolution of taxonomic relationships across fungal phylogenetic lineages, establishing them as the gold standard for accurate fungal classification.<sup>11</sup> Despite these advancements, coprophilous fungi remain underexplored compared to fungi from other substrates, such as plants, soil, or animals. Moreover, most fungi reported from this source have been identified solely based on morphological observations of the reproductive structures of the specimens directly growing

on the substrate. Molecular data are often lacking, as most of the studied taxa have not been successfully isolated. Due to the difficulty of differentiating species and even genera based on morphological data alone, reports on coprophilous fungi should be treated with caution unless supported by polyphasic studies. A typical example is the genus *Alternaria*, for which only four species were previously reported from dung worldwide, *i.e.* *A. alternata*, *A. atra*, *A. botrytis* and *A. chartarum*.<sup>12</sup> However, recent studies from samples collected solely in Spain, based on both morphological and molecular data derived from five different loci, identified 23 different species, including nine newly described species.<sup>13,14</sup> While the new taxa have not been reported in other substrates to date suggesting preference in growing in dung, the most frequent species found, *i.e.* *A. alternata*, *A. botrytis* and *A. atra*, are ubiquitous fungi.

As mentioned above, the isolation of this group of fungi has been uncommon in the past. We hypothesize that the main challenge lies in the difficulty of isolating interesting fungi besides common ubiquitous species. In the laboratory, fungal development in dung is induced using for instance the moist chamber technique, which consists of incubating samples under moist conditions by adding damp filter paper or paper towelling, under ambient light and at room temperature.<sup>9</sup> The moist chamber technique is a fundamental tool for favouring the development of dung-inhabiting fungi under laboratory conditions, and its introduction in the 1940s marked a milestone in their study. In fact, the increase in publications on this fungal group coincided with the widespread adoption of this technique in the 1960s.<sup>15</sup>



Fig. 1 Coprophilous fungi growing in different types of herbivorous dung. (A) *Pilobolus* sp. (Mucoromycotina) on horse dung. (B) *Triangularia setosa* (Ascomycota) on rabbit dung. (C) *Albifimbria viridis* (Ascomycota) on rabbit dung. (D) *Cephalotrichum* sp. (Ascomycota) on sheep dung. (E and F) Coprionoid fungi (Basidiomycota) on alpaca and giraffe dung, respectively. Scale bar: B = 100  $\mu$ m; C, D = 500  $\mu$ m; E, F = 1 mm.



However, the study of coprophilous fungi has been neglected in several countries, especially those across the African continent and other low-to-middle income countries.<sup>15</sup> One contributing factor is the implementation of the Nagoya Protocol, which restricts the exploitation of biological resources from signatory countries. Even the use of the biodiversity isolated by these countries is limited to institutions that have the necessary permits, such as Material Transfer Agreements (MTAs), which can be challenging to obtain and time-consuming to secure. Nonetheless, as coprophilous fungi represent an untapped resource of fungal diversity, significant knowledge gaps remain to be explored. Addressing these gaps could pave the way for future studies that not only expand our understanding of the diversity of these fungi, but also unlock their potential applications for society.

### 3. Bioactive secondary metabolites from coprophilous fungi

One of the many beneficial applications of dung-inhabiting fungi lies in their potential to produce novel natural products with therapeutic relevance.<sup>4</sup> The intricate complexity and limited knowledge surrounding coprophilous fungi make their study an exciting field but also highlight their promise for the discovery of biologically active SMs. Despite the few efforts to explore their chemistry, fungi isolated from dung have proven to be a rich reservoir of natural products with diverse bioactivities. This section presents examples of secondary metabolites from coprophilous fungi originating from various biosynthetic pathways, including polyketides, amino acid-derived compounds, terpenes, and hybrids. For each compound, details of its isolation, biological properties, biosynthesis, and, where available, total synthesis are discussed. Additionally, the supplementary information† includes a list of other SMs for which limited data is available beyond their isolation reports, along with brief descriptions of the producer organisms and their reported biological properties.

#### 3.1. Polyketide pathways

Polyketide synthases (PKSs) are multifunctional enzymes responsible for the biosynthesis of both life-saving molecules, such as the cholesterol-lowering drug lovastatin and the fungicidal strobilurins, as well as life-threatening metabolites, including some of the most devastating toxins like aflatoxins. In fungi, polyketides are predominantly synthesized by iterative type I PKSs, which can be classified into non-reducing (NR), partially reducing (PR), and highly reducing (HR) PKSs based on their catalytic domain composition and the degree of reduction in the resulting polyketide backbone.<sup>16,17</sup> Additionally, while less common, some species also harbour type III PKSs, which differ from type I and type II PKSs by utilizing free coenzyme A (CoA)-linked thioester substrates in an acyl carrier protein (ACP)-independent manner.<sup>16</sup> PKS-derived SMs account for the most abundant class of NPs, and several molecules of this class ( $n = 101$ ) have also been reported from dung-inhabiting species (Table S1†).

Analogous to the extensive chemical diversity within this class of NPs, these compounds exhibit a broad range of biological activities with potential applications in medicine and beyond. One example is the decalin sphingolipid synthesis inhibitor australifungin (**1**), which was isolated alongside australifunginol from *Sporormiella australis* (Sporormiaceae, Dothideomycetes), found in the dung of *Alces alces* in the USA.<sup>18,19</sup> In its original publication, this metabolite was reported as a potent antifungal agent, with MIC values below  $1 \mu\text{g mL}^{-1}$ , and its mode of action was linked to the inhibition of sphingolipid synthesis by preventing ceramide formation. Notably, australifungin was the first nonsphingosine-based inhibitor of sphingolipid biosynthesis, which motivated further studies and efforts toward its total synthesis.<sup>20–22</sup> This compound has a terminal  $\beta$ -ketoaldehyde, which is key to its potency, as its alcohol congener displays diminished biological effects. In fact, this functional group represents an unusual biosynthesis, since it likely involves a HRPKS terminating with an alternative domain similar to its biosynthetic relative, betaenone C (**2**), whose BGC encompasses Bet1, a HRPKS with an R releasing domain.<sup>23</sup> However, to the best of our knowledge, the BGC responsible for australifungin production remains unidentified and its total synthesis remains challenging due to the intricate assembly of both the  $\beta$ -ketoaldehyde and the  $\alpha$ -diketone functional groups (Fig. 2A).

Preussomerins denote a family of aromatic bis-ketals originally discovered from the coprophilous fungus *Preussia isomera* (Sporormiaceae, Dothideomycetes). Their structures were elucidated by extensive NMR experiments and X-ray crystallography.<sup>24</sup> Initially reported as potent antibacterials against *Bacillus subtilis* and *Staphylococcus aureus*, some derivatives also inhibited coprophilous species, such as *Ascobolus furfuraceus*.<sup>25</sup> These metabolites can be classified as spirobisanththalenes, as they feature a 1,8-dihydroxynaphthalene (DHN)-derived spiroketal unit linked to a second, oxidized naphthalene moiety. In general, this class of SMs display a wide range of biological properties, including anticancer, antimicrobial, and herbicidal properties. Palmarumycins represent a major subclass and are likely biosynthetic precursors to more complex derivatives. Their interesting chemistry and potential applications motivated total synthesis efforts as well as research on their elusive biosynthetic origin.<sup>26,27</sup> Their work revealed that the biosynthesis of palmarumycins, such as palmarumycin PCP<sub>1</sub> (**3**) requires the action of a physically distant PKS separate from the main biosynthetic gene cluster, which itself encodes only for two cytochrome P450s and a short chain dehydrogenase/reductase, but lacks major megasynthases (Fig. 2B). While synthesis of preussomerin G (**4**) from **3** has been achieved, the enzymatic steps to more complex spirobisanththalenes are yet to be elucidated.

Another example of unusual chemistry from dung-inhabiting fungi is found in the metabolites of *Delitschia confertasporea* (Delitschiaceae, Dothideomycetes), originally isolated from a sample of rock hyrax dung collected in Namibia. This species led to the discovery of delicoferones A and B, along with fimetarone A (**5**) and B (**6**).<sup>28</sup> Delicoferones possess a highly unusual skeleton, consisting of three aromatic rings linked *via*



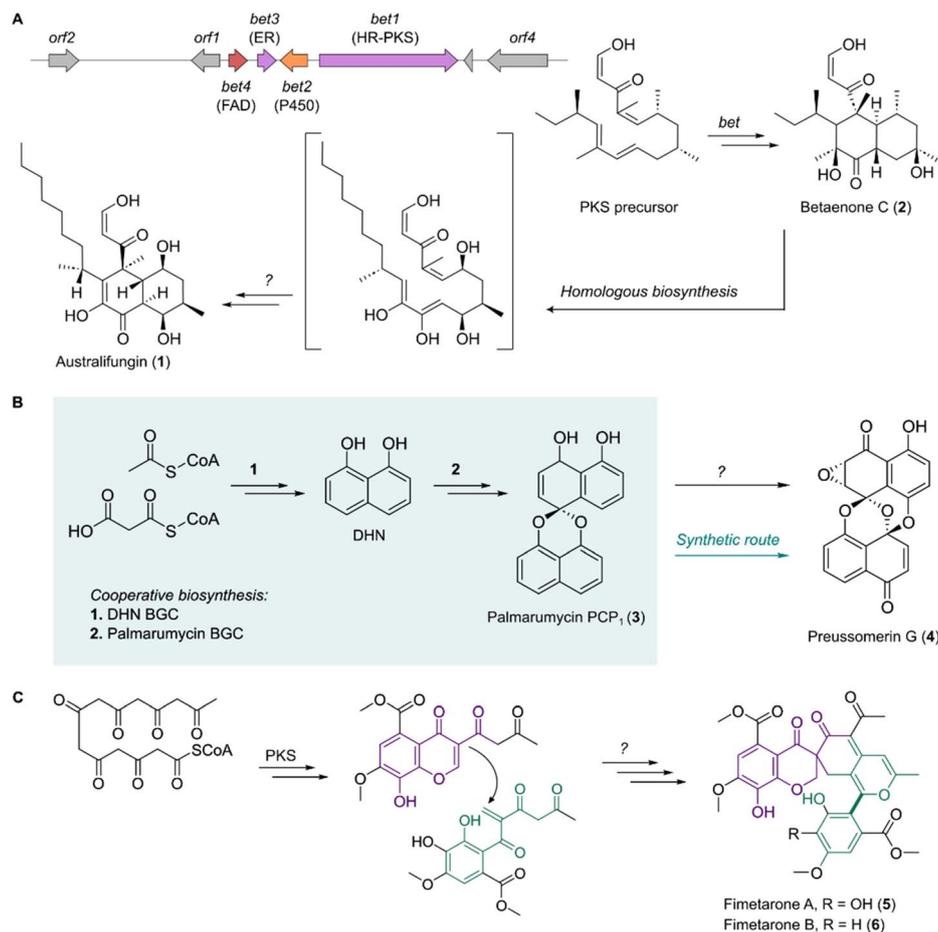


Fig. 2 Chemical structures of the different PKS-derived natural products from coprophilous fungi. (A) Australifungin (1) from *Sporormiella australis*, alongside the corresponding BGC for the homologous betaenone C (2) from *Neocamarosporium betae*. (B) Biosynthetic scheme for palmarumycin C (3) in *Berkleasium* sp., involving the coordinated action of two PKSs; palmarumycins serve as precursors for preussomerins, for which the total synthesis of preussomerin G (4) has been achieved. (C) Hypothetical biosynthetic pathway for fimatarone A (5) and B (6), suggesting its assembly from two distinct PKS-derived precursors.

two ketone carbonyl groups, while NMR analysis revealed structural similarities to fimatarone A, an uncommon metabolite featuring a spiro[chroman-3,7'-isochromene]-4,6'(8'H)-dione core.<sup>29</sup> Delicoferones A, B, and fimatarones appear to originate from a pseudodimeric biosynthetic assembly, likely formed by the fusion of two polyketide-derived subunits, as depicted in Fig. 2C. However, no further studies have been conducted on these secondary metabolites, despite their highly unusual carbon skeleton, which remains a rare structural motif among fungal natural products.

### 3.2. Amino acid derived pathways

Nonribosomal peptide synthetases (NRPSs) are modular multi-enzymes that synthesize amino acid-derived molecules.<sup>30</sup> Each module selects, activates, and incorporates an amino acid using adenylation (A), thiolation (T), and condensation (C) domains. The A domain activates the substrate, transferring it to the T domain to form a thioester linkage, while the C domain mediates peptide bond formation between adjacent modules. NRPS-like enzymes share the catalytic domains found in NRPS but

lack the condensation domain necessary for peptide formation. Several NRPS-derived products ( $n = 56$ ) have been reported from coprophilous fungi including different cyclic peptides, peptaibol-type linear oligopeptides, diketopiperazines, and other NRPS-like enzymes products (Table S2†).

NRPS-derived diketopiperazines constitute a group of diverse natural products widely produced by fungi, which often possess a complex core structure and display various biological activities.<sup>30</sup> For instance, the okaramines are one of the most unusual and structurally complex diketopiperazines.<sup>31</sup> These secondary metabolites possess potent insecticidal properties, explained by its selective activation of glutamate-gated chloride channels (GluCl) in a similar manner as the antiparasitic ivermectins.<sup>32</sup> Remarkably, okaramine B represents a lead compound targeting a ligand-gated ion channel found only in invertebrates. Several okaramine derivatives have been isolated from coprophilous fungi. For instance, the fungus *Aphanoascus fulvescens* (Eurotiomycetes) isolated from goose dung was found to produce the okaramines A–D (7–10), G, H, J, and V–Y, and Z (11).<sup>33</sup> Although the total synthesis of several okaramines was



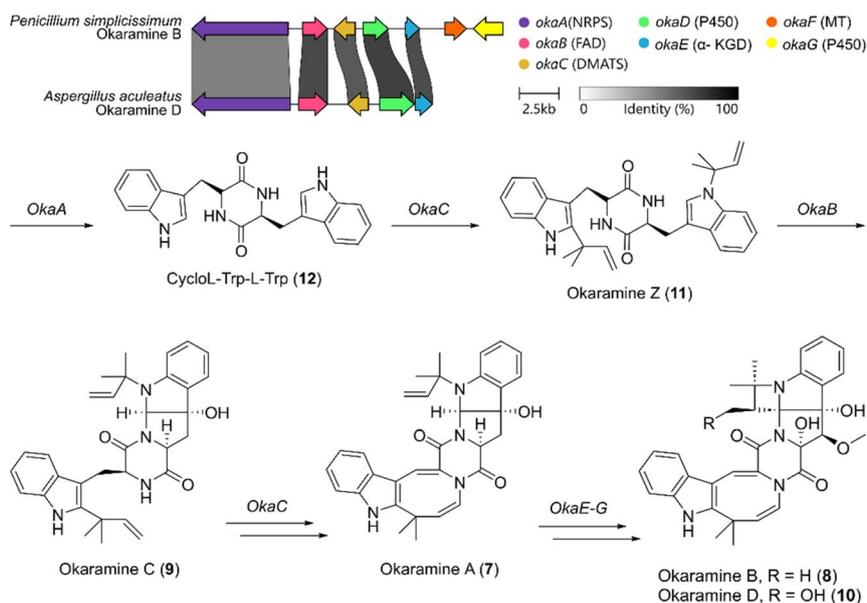


Fig. 3 Chemical structures of different okaramines from *Aphanoascus fulvescens* and comparative synteny analysis of the BGCs identified for okaramine B (8) and D (10) from *Penicillium simplicissimum* and *Aspergillus aculeatus*, respectively.

achieved early after their discovery, the synthesis of derivatives containing the four-membered azetidine ring has not been successful. In parallel, Lai *et al.* shed light on the biosynthetic basis for the most complex okaramines as illustrated in Fig. 3.<sup>31</sup> The diketopiperazine precursor of the okaramines pathway, cyclo-L-Trp-L-Trp (12), was isolated together with other by-products of the pathway, namely cyclo-(6 $\alpha'$ - $\alpha$ , $\alpha$ -dimethylallyl-L-Trp)-L-Trp, cyclo-(N8- $\alpha$ , $\alpha$ -dimethylallyl-L-Trp)-L-Trp, cyclo-(N8- $\alpha$ , $\alpha$ -dimethylallyl-L-Trp)-(6 $\alpha'$ - $\alpha$ , $\alpha$ -dimethylallyl-L-Trp).<sup>33</sup>

Other examples of diketopiperazines produced by coprophilous fungi include leptosin C (13) and the emestrins, which have been isolated from the Sordariomycetes members, *Preussia typharum* and *Podospora australis* (its taxonomic status remains uncertain as molecular data suggests its placement within the genus *Cladorrhinum*), respectively (Fig. 4).<sup>34,35</sup> Leptosins were first discovered from a strain of *Leptosphaeria* (Leptosphaeriaceae, Dothideomycetes) isolated from the marine alga *Sargassum tortile*. Generally these compounds contain at least one valine residue (leptosins A–K), a unique feature among all families of epi-polythiodiketopiperazines. Despite the fact that leptosins share several features with the verticillins, gliocladins, and chetracins, leptosins I and J present a C12–C11' ether linkage, which reduces the degrees of freedom of the molecule by the introduction of an additional ring. Nevertheless, these compounds have also been found in other ascomycetes, including members of the Aspergillaceae (Eurotiomycetes). These macrocyclic compounds are likely formed from two L-phenylalanine units by a peptide cyclization pathway similar to that of gliotoxin, an epidithiodiketopiperazine featuring a highly functionalized hydroindole scaffold known as an important mycotoxin produced by *A. fumigatus*.<sup>36</sup> However, in the case of the emestrins, the cyclization is followed by additional ring-expansion and further macrocyclization steps.<sup>34</sup>

Both leptosins and emestrins have been extensively characterized by their potent cytotoxic effects on different mammalian cell lines.<sup>34,35</sup> However, it has also been shown that some of these epidithiodiketopiperazines exhibit selective anti-fungal effects as in the case of 13 and emestrin C (14) against the pathogenic yeast *Cryptococcus neoformans*.<sup>35</sup> The mechanism of the selective action of these compounds remains uncertain, since ATP synthesis and mitochondrial function are conserved features between fungi and other eukaryotic organisms. Even though the biosynthesis of epi-polythiodiketopiperazines is very well understood, as in the case of the okaramines, further studies are needed in the case of emestrin-like molecules.

Terezines A–C, and D (17) were also found to be produced in liquid cultures of *Sporormiella teretispora* (Sporormiaceae, Dothideomycetes). Compounds A–C originate from the modification of a diketopiperazine formed from valine and phenylalanine, while terezine D is derived from alanine and a prenylated tryptophan unit, and is in fact a shunt product during the biosynthesis of hexadehydroastechrome (18) (Fig. 5A).<sup>37</sup> Terezines exhibited weak inhibition of Gram-positive bacteria and moderate inhibition of other coprophilous fungi such as *Sordaria fimicola* and *Ascobolus furfuraceus* in disk diffusion assays.<sup>38</sup> Benzomalvin A/D (19/20), quinolactacins A1, A2 and B, quinolonimide, and asperphenamate were isolated from solid culture of *Penicillium spathulatum* (Aspergillaceae, Eurotiomycetes). Benzomalvin A/D as well as the quinolactacins were investigated for their  $\alpha$ -glucosidase inhibition properties, demonstrating the first *in vivo* inhibition of this enzyme in normal and hyperglycemic mice.<sup>39</sup> Benzomalvins have also been demonstrated to inhibit the human NK1 receptor, hampering the effects of substance P, a neurokinin peptide involved in pain transmission and inflammation.<sup>40</sup> Similarly, these compounds antagonize the human enzyme 2,3-indoleamine dioxygenase,



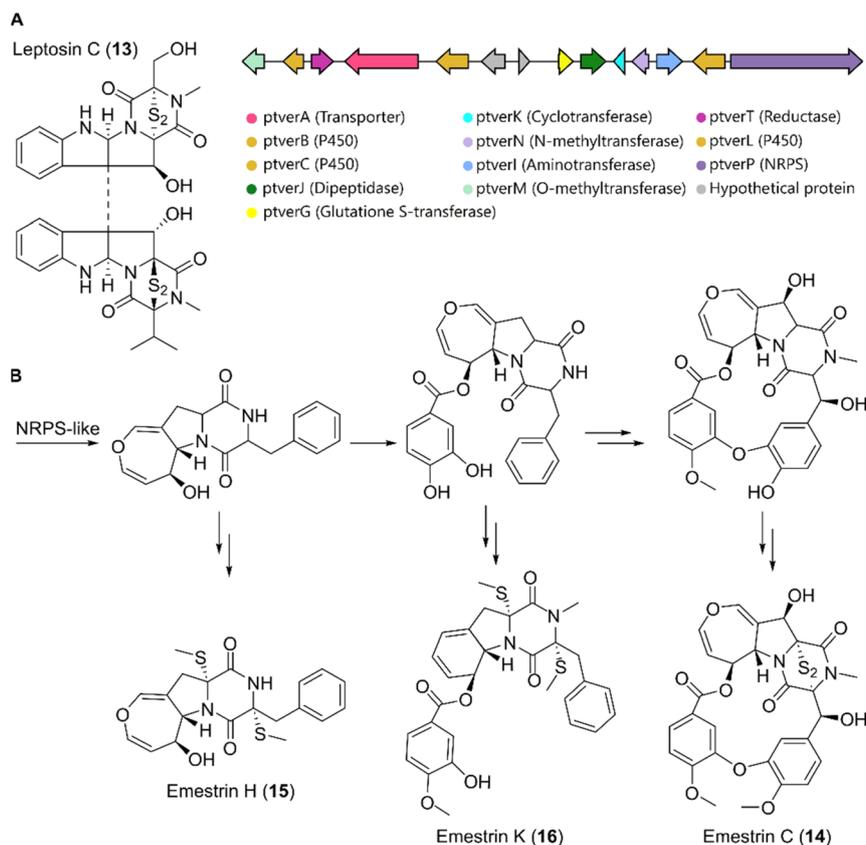


Fig. 4 (A) Chemical structure of leptosin C (13) from *Preussia typharum* and its corresponding BGC. (B) Hypothetical biosynthetic pathway for emestrins, produced by *Podospora australis*.

a potential target for the development of therapies for pathologies ranging from autoimmune disorders to Alzheimer's disease.<sup>41</sup> The benzomalvin biosynthetic gene cluster consists of three genes: a putative SAM-binding methyltransferase benX and two NRPS genes benY and benZ (Fig. 5B). This discovery was driven by using fungal artificial chromosomes with metabolomic scoring (FAC-MS), identifying the terminal cyclizing condensation domain as BenY-CT and the internal C-domains as BenZ-C1 and BenZ-C2.<sup>42</sup> Moreover, the evidence suggested that BenY-CT or an unidentified protein facilitates the benzodiazepine formation, representing the first reported benzodiazepine synthase enzymatic activity.

Malbrancheamide, malbrancheamide B (21), iso-malbrancheamide B, and pre-malbrancheamide (22) are unusual indole alkaloids possessing a bicyclo [2.2.2] diazoctane core isolated from *Malbranchea aurantiaca* (Onygenaceae, Eurotiomycetes).<sup>43</sup> These metabolites are related to the brevianamides and, like the aspergamides, marcfortines, paraherquamides and sclerotamides, are biosynthesised from tryptophan, proline or lysine and at least one isoprene unit. Despite the early idea that the monoketopiperazines (MKP) and diketopiperazines (DKP) shared a common biosynthetic origin; it was later shown that two divergent types of intramolecular Diels–Alderses operate to generate the MKP and DKP ring systems of these metabolites.<sup>44</sup> The malbrancheamide and paraherquamide gene clusters lack homologous genes that

encode known Diels–Alderses (Fig. 5C), and just recently it was established that these two homologous systems function through a bifunctional reductase and a Diels–Alderase that evolved from an ancestral short-chain dehydrogenase (SDR) and is also encoded in several other fungal natural product biosynthetic gene clusters.<sup>45</sup> This biosynthetic divergence also raises the question of whether compounds such as flutimide, which feature an unusual 1,3-diketo arrangement rather than the more common 1,4-diketo configuration, are produced *via* a yet unknown biosynthetic logic.<sup>46</sup>

### 3.3. Terpene pathways

Terpenes are synthesized through the assembly of dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), which originate in nature *via* the mevalonate (MVA) or methylerythritol 4-phosphate (MEP) pathways. In fungi, terpene biosynthesis relies exclusively on the MVA pathway.<sup>47</sup> Similar to polyketide biosynthesis, the MVA pathway requires acetyl-CoA units for the synthesis of mevalonate, a key intermediate that, after a series of downstream reactions, yields IPP and its isomer DMAPP. These two isomers exhibit distinct reactivity, with DMAPP acting as a C1 electrophile and IPP as a C4 nucleophile. Their sequential condensation forms geranyl diphosphate (GPP), which ultimately leads to the biosynthesis of squalene, a central precursor of triterpenes across all fungal lineages.<sup>47</sup> These metabolites represent



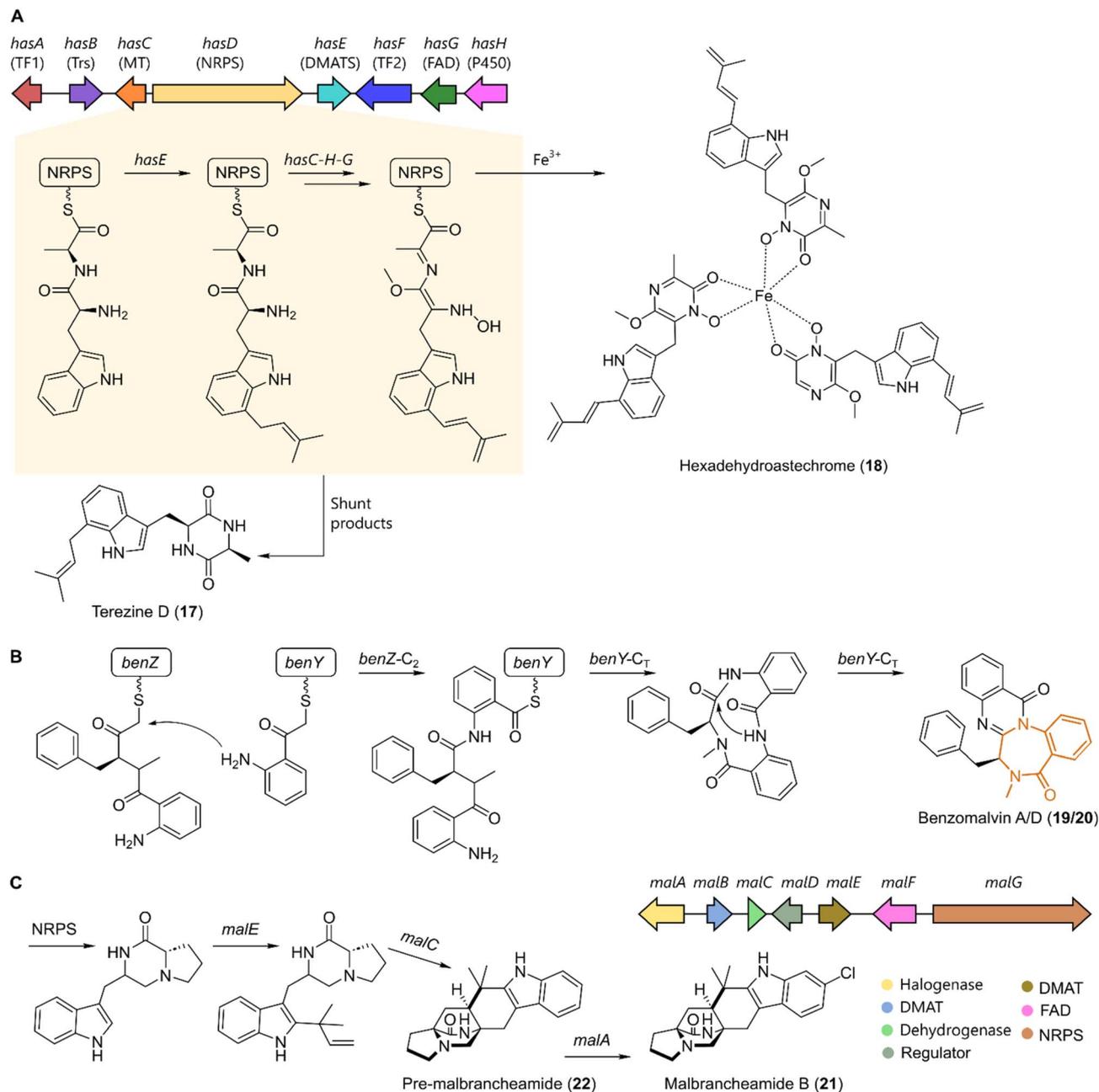


Fig. 5 (A) Chemical structures of terezine D (**17**), a shunt product in the biosynthesis of hexadehydroastechrome (**18**), along with its respective BGC. (B) Proposed biosynthetic pathway for the assembly of the benzodiazepine core of benzomalvin A/D (**19/20**). (C) Biosynthetic route for the production of bicyclo[2.2.2]diazaoctane indole alkaloids, such as malbrancheamides, along with their corresponding BGC.

one of the most common classes of NPs produced by these organisms, and are seemingly related to their chemical ecology. Many of them play roles in stress responses, protection of cell membrane integrity, UV protection, microbial interactions, as well as host growth and defense.<sup>48</sup> Diverse examples of terpene derived SMs ( $n = 36$ ) have been reported from dung-inhabiting fungi as summarized in Table S3.†

A prominent example is the furanosteroid class to which wortmannin (**23**) belongs, of which several derivatives were isolated from a *Niesslia* sp. (Niessliaceae, Sordariomycetes) obtained from horse dung. These compounds, along with

structurally related wortmannines that feature an unusual ring system, highlight the chemical diversity within this group.<sup>49</sup> Wortmannins, originally discovered in *Penicillium wortmanni* (Aspergillaceae, Eurotiomycetes) as selective antifungal agents, are well-known as potent phosphoinositide 3-kinase (PI3K) inhibitors, which motivated several efforts to achieve their total synthesis.<sup>50</sup> In fact, the semisynthetic derivative PX-866 progressed to phase II clinical trials for cancer treatment.<sup>51</sup> These secondary metabolites are derived from the steroid biosynthetic pathway through distinct oxidative steps that remove carbons from sterol precursors, following a biosynthetic route



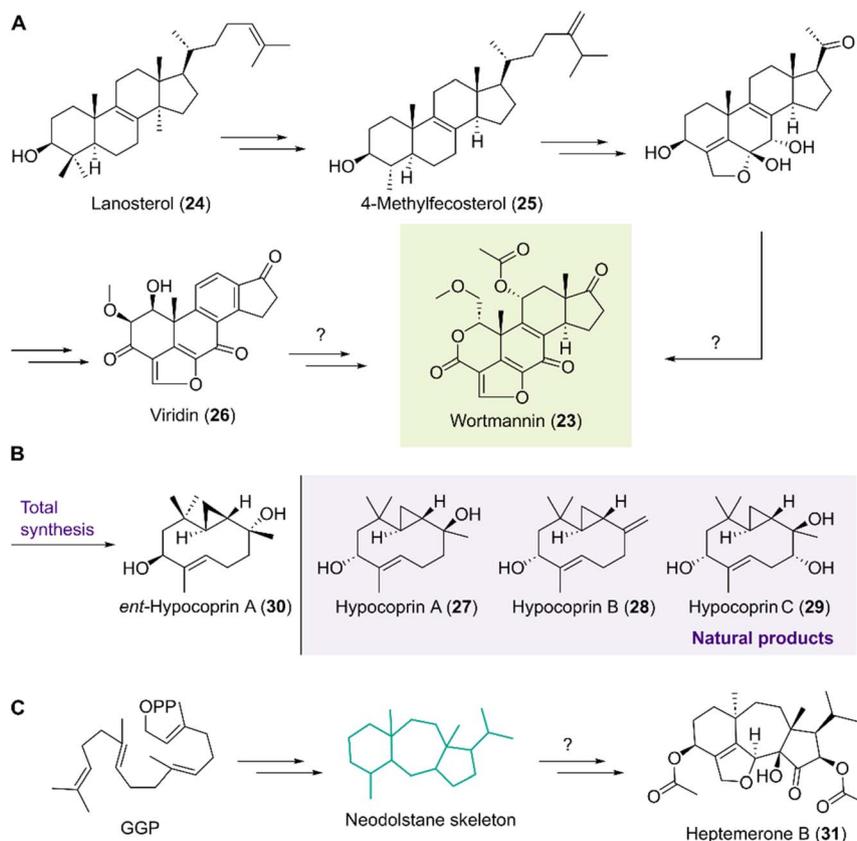


Fig. 6 (A) Biosynthetic pathway for the production of viridin (26), a furanosteroid related to wortmannin (23). (B) Chemical structures of the NPs hypocoprins A–C (27–29) and the synthetic *ent*-hypocoprin A (30). (C) Proposed biosynthetic pathway for the assembly of the neodolastane skeleton from geranylgeranyl diphosphate (GGP), which, after further tailoring, gives rise to heptemerone-like SMs.

homologous to that of viridin (26) (Fig. 6A).<sup>52</sup> Similarly, hypocoprins A–C (27–29) were isolated from *Hypocopra rostrata* (Xylariaceae, Sordariomycetes). These molecules possess a distinctive ring system consisting of fused cyclopropane and cyclodecene units. Hypocoprin A exhibited antibacterial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.<sup>53</sup> The unique 3/10 bicyclic sesquiterpenoid carbon skeleton of these compounds has attracted synthetic interest, aiming to expand access to this rare class of natural products. However, efforts to achieve their total synthesis have so far been unsuccessful, only achieving the synthesis of *ent*-hypocoprin A (30) (Fig. 6B).<sup>54,55</sup> Likewise, the diterpenoids heptemerones, produced by *Coprinus heptemerus* (Psathyrellaceae, Agaricomycetes), exhibited strong inhibition of fungal germination in *Pyricularia grisea*, the causative agent of rice blast disease and a major threat to rice cultivation worldwide.<sup>56,57</sup> Structurally, heptemerones share a tricyclic neodolastane carbon skeleton with guanacastepenes, characterized by two angular methyl groups in a 1,4 relationship at C8 and C11 and an additional isopropyl substituent at C12. Although both guanacastepenes and heptemerones feature a nonpolar, unfunctionalized “upper rim,” they are distinguished by differences in oxygenation and unsaturation patterns on the “lower rim” of the molecules.<sup>58</sup> Due to their intriguing structures and promising biological activities, these compounds

have been motivation for different total synthesis campaigns.<sup>58,59</sup> Both heptemerones and guanacastepenes are hypothesized to originate from geranylgeranyl diphosphate (GGP) *via* a series of enzyme-catalyzed ring closures and Wagner–Meerwein migrations (Fig. 6C). However, to date, no targeted biosynthetic studies have been conducted on this class of molecules, highlighting the need for future efforts to elucidate their biogenesis.

### 3.4. Hybrid pathways

As discussed in previous sections, the biosynthesis of fungal NPs is a complex process and represents a rapidly growing field of research. Beyond the previously covered classes of SMs pathways, there are instances where two or more molecules, originating from separate pathways, are combined to form a single natural product. This process, known as convergent biosynthesis, adds to the intricate chemical complexity and structural diversity of fungal metabolites.<sup>60</sup> In other cases, such as with iterative hybrid polyketide synthases–nonribosomal peptide synthetases (PKS–NRPS), the domains from different pathways are fused together. This combination results in the assembly of a nascent polyketide chain and an amino acid in an intra-enzymatic manner.<sup>61</sup> Interestingly, it is also possible that collaborative biosynthetic machineries may be present,



meaning that the respective core enzymes are not fused, but encompassed within the same BGC.<sup>60</sup> Coprophilous fungi are indeed creative SM producers and their enzymatic machineries have evolved in such ways, resulting in intriguing SMs originating from a mixed biosynthetic background ( $n = 6$ ) (Table S4†).

A prominent example is *Areothea areolata* (Naviculisporaceae, Sordariomycetes), isolated from porcupine dung, which produces potent trichothecene toxins such as roridin E (32). This molecule exhibited the ability to inhibit early successional coprophilous fungi, including *S. fimicola* and *Ascobolus furfuraceus*.<sup>62</sup> Roridin E belongs to the trichothecene-like toxins, a class of mycotoxins widely produced by various ascomycetes. Macrocyclic trichothecenes are characterized by a macrocyclic ring formed through the esterification of a linear polyketide substituent at C4 of the 12,13-epoxytrichothec-9-ene core. Additionally, an isoprenoid substituent is esterified at C15 of the same core, and an ether bond links the polyketide and isoprenoid substituents.<sup>63</sup> In fact, the absence of the macrocyclic structure in such trichothecenes has been demonstrated to alter significantly the biological activities of these SMs, which are hypothesized to be linked to the ecological role of these toxins (Fig. 7A).

A notable class of PKS-NRPS hybrid products includes tetramic acids and their 2-pyridone congeners, which exhibit a vast diversity throughout diverse fungal lineages.<sup>64,65</sup> The coprophilous fungus *Apiospora montagnei* (Apiosporaceae, Sordariomycetes), isolated from mouse dung, was found to produce the antifungal apiosporamide (35). This compound exhibited antifungal activity against the early successional coprophilous fungus *Ascobolus furfuraceus* and demonstrated antibacterial activity, forming inhibition zones against *Bacillus subtilis* and *Staphylococcus aureus*.<sup>66</sup> The complexity of its planar structure and stereochemistry, combined with its potent biological properties, has driven efforts to develop synthetic alternatives to resolve ambiguities in its stereochemical configuration and generate related analogs with stereodivergent properties that may influence its biological activity.<sup>67</sup> While analogous BGCs have been reported that may be responsible for production of 35, no targeted studies have been conducted to elucidate the molecular mechanisms and enzymatic machinery involved in its biosynthesis.<sup>68</sup> Nevertheless, it is expected to follow biosynthetic steps homologous to those of related SMs, such as fischerins and sambutoxins (Fig. 7B).

The meroterpenoids ascochlorin (37) and 5-chlorocolletorin B have been reported from the coprophilous fungus

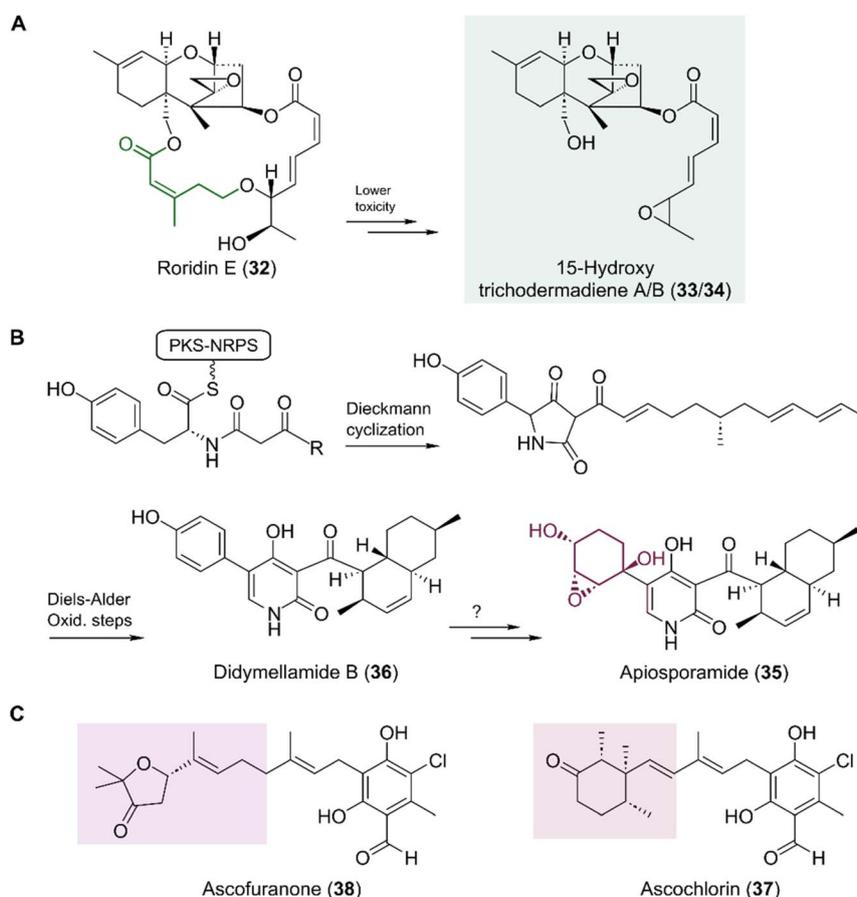


Fig. 7 (A) Chemical structures of roridin E (32) and its biosynthetic precursor 15-hydroxy trichodermadiene A/B (33/34). (B) Biosynthetic scheme for the production of apiosporamide (35) from a didymellamide-like precursor. (C) Chemical structures of ascofuranone (38) and ascochlorin (37), which represent an interesting example of branching biosynthesis.



*Hapsidospora globosa* (syn. *Nigrosabulum globosum*; Incertae sedis, *Sordariomycetes*), isolated from sheep dung in Australia.<sup>69</sup> These secondary metabolites exhibit strong antimicrobial activity, while the related ascofuranone (**38**) has emerged as a promising drug candidate for cancer, alveolar echinococcosis, and African trypanosomiasis. The latter disease, caused by *Trypanosoma brucei*, relies on trypanosome alternative oxidase (TAO) for energy metabolism, which ascofuranone potently inhibits, making it a potential therapeutic agent. The biosynthetic origin of both ascochlorin and ascofuranone has been elucidated in *Acremonium egyptiacum* (syn. *Acremonium sclerotigenum*; Bionectriaceae, *Sordariomycetes*).<sup>70–72</sup> Both compounds share the common precursor ilicicolin A epoxide, which is cyclized by *AscF* in ascochlorin biosynthesis. In contrast, ascofuranone biosynthesis branches off through hydroxylation at C-16 by the P450 monooxygenase *AscH*, followed by cyclization *via* the terpene cyclase *AscI* (Fig. 7C). The genes required for ascochlorin biosynthesis and its transcriptional regulator form a single BGC, whereas those involved in the late steps of ascofuranone biosynthesis are located in a separate, distantly positioned cluster.<sup>70,73</sup>

## 4. Biotechnological applications

The previous sections have highlighted the biosynthetic creativity of coprophilous fungi in producing anti-infective SMs and other potentially beneficial molecules. However, beyond their role as prolific SMs producers, these fungi also possess remarkable enzymatic capabilities that make them valuable for biotechnological applications. Their adaptation to herbivore dung, a nutrient-limited, highly competitive environment, has driven the evolution of specialized enzymes capable of breaking down complex organic matter, including recalcitrant lignocellulosic biomass and other plant residues.<sup>3,74</sup> These biocatalysts are of great interest in various industries, particularly in biofuel production, bioremediation, and sustainable manufacturing. Despite their clear potential, research into the industrial applications of coprophilous fungi remains limited, with only a few studies exploring their enzymatic versatility. On the other hand, coprophilous taxa such as *Triangularia anserina* (syn. *Podospora anserina*) or *Sordaria fimicola* are model organisms due to their remarkably rich genomes dedicated to the catabolism of complex biopolymers.<sup>75</sup>

Lignocellulolytic enzymes from coprophilous fungi, including laccases, peroxidases, cellulases, hemicellulases, and pectinases, are promising for various biotechnological applications.<sup>74,76,77</sup> These enzymes enable biofuel production by breaking down lignocellulosic biomass into fermentable sugars, supporting second-generation bioethanol and biogas. They also degrade pollutants like polycyclic aromatic hydrocarbons, synthetic dyes, pesticides, and microplastics, making them valuable for bioremediation. In the paper and textile industries, fungal oxidases and hydrolytic enzymes aid in bio-bleaching and fiber processing, reducing chemical use. Studies on coprophilous fungi from koala feces identified high-yield producers like *Neurospora cratophora* (Sordariaceae, *Sordariomycetes*) and *Trichoderma atroviride* (Hypocreaceae,

*Sordariomycetes*), which produce heat-tolerant enzymes, while *Cephalotrichum stemonitis* (syn. *Doratomyces stemonitis*; Microascaceae, *Sordariomycetes*) produces hemicellulases, endoglucanases, and  $\beta$ -glucosidases with neutral to alkaline pH optima. Additionally, *Mariannaea camptospora* (Nectriaceae, *Sordariomycetes*) secretes cold-tolerant lipases.<sup>76</sup> These enzymes are highly sought after for industries such as paper, detergents, and food products, and could be further optimized through strain improvement programs, driving greener, sustainable technologies.

## 5. Future perspectives and challenges

Coprophilous fungi denote a rich reservoir of structurally complex and biologically active SMs, many of which likely contribute to the enhanced ecological fitness they need to survive in such competitive substrates. Throughout this review, we have discussed numerous examples of molecules produced by these fungi and originating from different biosynthetic classes, including polyketides, nonribosomal peptides, terpenes, and hybrid metabolites, emphasizing their potential for antimicrobial discovery and other applications. Despite their ecological significance and chemical potential, the study of coprophilous fungi has been geographically biased, with significant gaps in knowledge, particularly in regions such as Africa and South America.<sup>15</sup> Expanding biodiversity surveys in these areas could lead to the discovery of new fungal taxa. A potential strategy to uncover the hidden diversity of dung-inhabiting taxa is the use of selective isolation methods that favor the growth of uncommon fungal groups. For instance, we adapted ascospore activation techniques involving heat and chemical treatments to process dung samples, leading to promising preliminary results.<sup>78</sup> These methods selectively kill spores of common molds such as *Mucor*, *Penicillium*, and *Aspergillus*, while activating resting spores and promoting germination of teleomorphic stages, particularly for ascomycetes. Teleomorphs typically grow slower than their respective anamorphs and are therefore less often isolated and studied. This technique might improve the isolation of diverse fungal taxa, beyond the common generalists typically obtained using moist chambers. We believe that this can increase the chances of isolating novel strains that can be included in the screening pipeline of drug discovery laboratories without the need for specialized expertise in fungal taxonomy. As previously demonstrated in other groups of organisms, such as myxobacteria, the chances of finding new chemical entities are greater by examining untapped lineages rather than already studied ones.<sup>79</sup> Therefore, expanding the known diversity of fungi, particularly from underexplored ecological niches such as dung, as discussed herein, is essential to fuel the discovery of novel antimicrobial agents.

As our understanding of coprophilous fungal ecology advances, novel isolation and cultivation techniques will be essential for fully exploiting their biotechnological potential. Many species likely remain uncultured because standard approaches to fungal cultivation fail to replicate their natural environmental conditions, limiting our ability to study them



under laboratory conditions. Artificial intelligence and machine learning approaches could provide valuable insights into the growth requirements of these cryptic fungi.<sup>80</sup> By leveraging expanding genomic datasets, these technologies could help predict relationships between specific nutrient conditions and secondary metabolism, ultimately guiding the development of optimized culture strategies to unlock their biosynthetic potential.<sup>81</sup> From this perspective, the remarkable structural diversity observed in coprophilous fungi suggests the presence of highly specialized enzymatic machineries, yet only a handful of BGCs have been characterized from coprophilous fungi, and in the cases where they were studied, the genome sequences are mostly from a different producer rather than from the dung-inhabiting fungus.<sup>82</sup> However, for most of the bioactive SMs discussed, their biosynthetic origins remain unknown. Addressing this gap could provide insights into novel enzymatic reactions and expand the chemical space available for drug discovery. Interestingly, the ecological role of many of these metabolites remains poorly understood. While some compounds, such as sordarins and antiameobins, appear to provide competitive advantages during dung colonization, the selective bioactivity of other SMs suggests alternative ecological roles.

Future studies should focus on untangling these ecological functions, as they may provide clues for optimizing the production of specific metabolites for biotechnological applications. Particularly, species belonging to the Sordariomycetes, Dothideomycetes, and Eurotiomycetes are frequently encountered in dung and these taxa are prolific producers of SMs with fascinating chemical structures and significant biological activities. These findings contribute to advancing our understanding of the ecological roles of genera within these diverse lineages. For example, taxa within the Sordariales have already proven to be an untapped reservoir of innovative producers, yet entire genera, and even families, remain unexplored.<sup>83</sup> Overall, this review underscores the potential of coprophilous fungi as a valuable source of bioactive NPs and highlights the need for multidisciplinary approaches that integrate taxonomy, genomics, and ecological studies. By filling the existing knowledge gaps and leveraging advances in OMICs technologies, we can better understand the biosynthetic potential of these fungi and accelerate the discovery of new antimicrobial and therapeutically important molecules.

For instance, the concurrent systematic study of the Xylariales, a fungal order closely related to the Sordariales, which are predominant in dung, serves as a compelling example of how the targeted exploration of biological and chemical diversity within a defined lineage can lead to the discovery of biologically active natural products. Over the past decade, several taxa within this order have been cultured and studied to refine their taxonomic placement and to gain insights into their secondary metabolism. A pilot phylogenomic study employing third-generation sequencing technologies (PacBio and Oxford Nanopore) on 13 representative strains has opened new avenues for further research on their ecology, evolution, and biosynthetic diversity.<sup>84,85</sup> Presently and partly due to these efforts, more than 100 high-quality genomes are available, enabling comparative

OMICs and facilitating synthetic biology campaigns, including total biosynthesis of selected natural products and targeted metabolome mining of interesting candidates.<sup>86,87</sup> As genome sequencing efforts for the Sordariales continue to expand, we anticipate uncovering similarly rich metabolic potential within this ecologically and chemically promising fungal lineage.

## 6. Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

## 7. Author contributions

Writing—review & editing: EC-G, JT, MS, YMF; writing—original draft: EC-G, YMF; visualization: EC-G, JT, YMF; conceptualization: EC-G, MS, YMF; funding acquisition: YMF. All authors contributed to the article and approved the final submission.

## 8. Conflicts of interest

There are no conflicts to declare.

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