Ecological and evolutionary perspectives advance understanding of mycobacterial diseases

Christine Chevillon, Benoît De Thoisy, Alex Rakestraw, Kayla Fast, Jennifer Pechal, Sophie Picq, Loïc Epelboin, Paul Le Turnier, Magdalene Dogbe, Heather Jordan, et al.

Ecological and evolutionary perspectives advance understanding of mycobacterial diseases

Christine Chevillon, Benoît de Thoisy, Alex W Rakestraw, Kayla M Fast, Jennifer L Pechal, Sophie Picq, Loïc Epelboin, Paul Le Turnier, Magdalene Dogbe, Heather R Jordan, Michael W Sandel, Mark Eric Benbow†, Jean-François Guégan†*

Predicting the outbreak of infectious diseases and designing appropriate preventive health actions require interdisciplinary research into the processes that drive exposure to and transmission of disease agents. In the case of mycobacterial diseases, the epidemiological understanding of the scientific community hitherto was based on the clinical studies of infections in vertebrates. To evaluate the information gained by comprehensively accounting for the ecological and evolutionary constraints, we conducted literature searches assessing the role of mycobacteria interactions with non-vertebrate species in the origin of their pathogenicity and variations in disease risk. The reviewed literature challenges the current theory of person-to-person transmission for several mycobacterial infections. Furthermore, the findings suggest that diverse non-vertebrate organisms influence virulence, mediate transmission, and contribute to pathogen abundance in relation to vertebrate exposure. We advocate that an ecological and evolutionary framework provides novel insights to support a more comprehensive understanding of the prevention and management of diseases in vertebrates.

Introduction

Insights into mycobacterial ecology and evolution from the medical and veterinary perspectives can be divided into two categories.[1] The first category involves research on the *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium leprae,* and mycobacterial pathogens, without an environmental stage, which infect single vertebrate species. These pathogens are posited to be transmitted from person to person, although empirical evidence exists only for MTBC.[1] The second category focuses on non-tuberculous mycobacteria (NTM), which persist in the soil and freshwater as free-living saprophytes—organisms that use decomposing organic matter.[1] Susceptible vertebrates acquire NTMs from exposure to such environments,[1] suggesting ecological variations in the risk of acquiring a disease. Currently, there is little overlap between the historical research on MTBC and that on NTMs present in the environment, ignoring potential insights from the ecological and evolutionary perspectives of mycobacteria responsible for numerous diseases.

MTBC remains one of the deadliest agents of human infection, with only SARS-CoV-2 surpassing it.[2] WHO classifies leprosy (caused by *M leprae*) as a neglected tropical disease, and untreated leprosy infections can lead to permanent disability, deformity, and stigmatisation. Approximately 200 000 new cases of leprosy were reported in the year 2019, with 79% of them in India, Brazil, and Indonesia.[3] WHO also classifies Buruli ulcer (caused by an NTM, *Mycobacterium ulcerans*) as a neglected tropical disease; untreated Buruli ulcer can lead to permanent disability. In west Africa alone, the prevalence of Buruli ulcer ranges from 3 to 27 cases per a population of 10 000.[4] The prevalence of other NTMs is rising worldwide, with some local estimates currently at 3 per a population of 10 000 and predominantly resulting in pulmonary infections associated with morbidity and mortality in humans.[5] Despite the geographical variation in specific burdens, the main health concerns are related to *Mycobacterium absessus, Mycobacterium chelonae, Mycobacterium fortuitum, Mycobacterium avium* subspecies *avium*, *Mycobacterium avium* subspecies *intracellulare*, and *Mycobacterium kansaii*.[6] Understanding how mycobacterial traits mediating exposure to infections and pathogenicity have evolved over time and space should provide new insights into the determinants of mycobacterial disease burden.

Here are the definitions of some terms commonly used in ecological and evolutionary research on infectious diseases, which provide cross-disciplinary clarity when discussing the evolution of mycobacterial pathogenicity. Pathogen transmission between unrelated individuals is considered horizontal transmission, whereas that from parent to offspring is considered vertical transmission. A parasite is any pathogen with a parasitic lifecycle (horizontal transmission), whereas an inherited symbiont is a pathogen that moves by means of vertical transmission. The term host refers to the species that is part of the lifecycle of a parasite or an inherited symbiont. The harm caused by within-host replication of a pathogen has a genetic basis and reduces host fitness (lifetime offspring number) by a quantity that is considered as virulence. Many theoretical models have examined why parasites have not evolved to be benign since they depend on the hosts for their survival. A review of studies on these models indicated that they are all based on the adaptive virulence scenario (AVS).[7] The AVS posits that parasites evolve intermediate values of virulence to maximise their fitness (the number of secondary infections) and balance the cost of a reduction in the infectious period associated with benefits conferred by an increasing transmission rate throughout their lifecycle (figure 1).[7] Notably, for indirectly transmitted parasites, transmission includes survival in the environment or probabilities of infecting the intermediate host species and of onward transmission.[7,8] Upon considering the parasitic lifecycles in full, empirical studies have provided strong support for the AVS.[7,8]

Lancet Microbe 2024

Published Online
https://doi.org/10.1016/S2666-5247(24)00138-1

*Present affiliation: Field Museum of Natural History, Chicago, IL, USA
†Contributed equally

MIVEGEC, Université de Montpellier, Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement, Institut National de Recherches pour l'Agriculture, l'Alimentation et l'Environnement, Montpellier, France (C Chevillon PhD, Prof J-F Guégan PhD); **Laboratoire des Interactions Virus Hôtes, Institut Pasteur de la Guyane, Cayenne, French Guiana, France** (B de Thoisy PhD); **Department of Entomology** (A W Rakestraw MSc, J L Pechal PhD, S Picq PhD, Prof M E Benbow PhD), **Department of Osteopathic Medical Specialties** (Prof M E Benbow), **Ecology, Evolution and Behavior Program** (Prof M E Benbow), **and Agbioresearch** (Prof M E Benbow), **Michigan State University, East Lansing, MI, USA; Department of Wildlife, Fisheries, and Aquaculture** (K M Fast MSc, M W Sandel PhD), **Department of Biological Sciences** (M Dogbe MSc, H R Jordan PhD), **and Forest and Wildlife Research Center** (M W Sandel), **Mississippi State University, MS, USA; Unité des Maladies Infectieuses et Tropicales** (Prof L Epelboin MD PhD, P Le Turnier MD) **and Centre d'Investigation Clinique Antilles-Guyane, Inserm 1424** (Prof L Epelboin, P Le Turnier), **Centre Hospitalier de Cayenne, Cayenne, French Guiana, France; Epidémiologie des maladies animales et zoonotiques, Université Clermont Auvergne, INRAE, VetAgro Sup, Saint-Genès-Champanelle, France** (Prof J-F Guégan); **Epidémiologie des maladies animales et zoonotiques, Université de Lyon,**

INRAE, VetAgro Sup, Marcy l'Etoile, France (Prof J-F Guégan)

Correspondence to: Dr Christine Chevillon, MIVEGEC, Université de Montpellier, Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement, Institut National de Recherches pour l'Agriculture, l'Alimentation et l'Environnement, Montpellier 34 395, France
christine.chevillon@ird.fr

The particularity of inherited symbionts is that, in addition to virulence, they provide hosts with nutritional or defensive benefits, which are ecologically variable.[9] Therefore, the positive or negative net infection effect on host fitness fluctuates in time and space (figure 2A).[9] Thus, the hosts maximise their fitness by driving symbiont evolution towards an intracellular lifestyle and maximum vertical transmission (figure 2B).[9] By contrast, inherited symbionts maximise their fitness by retaining some horizontal transmission.[9] Overall, these systems result in facultatively inherited symbionts that are adapted to intracellular lifestyles and are regularly excreted by the hosts, opening up opportunities for spillovers to other species.[9] A good example is the evolution of *Rickettsia*, in which the bacteria recurrently shifted across diverse hosts (eg, protists, leeches, and arthropods) as inherited symbionts and repeatedly evolved from inherited symbionts to arthropod-borne parasites.[12]

The coincidental virulence scenario (CVS) refers to cases in which pathogenicity does not rely on the infection under study.[13] Two functional explanations can describe the pathogenicity of CVS. The first relies on spillover to a new species that shares, by chance, a similar susceptibility as the host(s) with which the pathogen has coevolved as a parasite or inherited symbiont. This explanation reflects human disease caused by the spillover of *Legionella pneumophila*, whose pathogenicity arose from its adaptation to protozoan hosts, following accidental introductions into human-engineered habitats.[14] The pleiotropy of virulence factors (ie, their involvement in several metabolic pathways affecting different phenotypes) is a second functional basis for the CVS; the selection of such genes can rely on phenotypic advantages unrelated to the infection under study. An experimental design of bacterial evolution using *Escherichia coli* and resistance to protozoan grazing as primary selection provided supporting evidence of this view not being merely a theoretical argument.[15] Otherwise, bacterial virulence factors often play a role in regulating interspecific competition.[16,17]

See Online for appendix

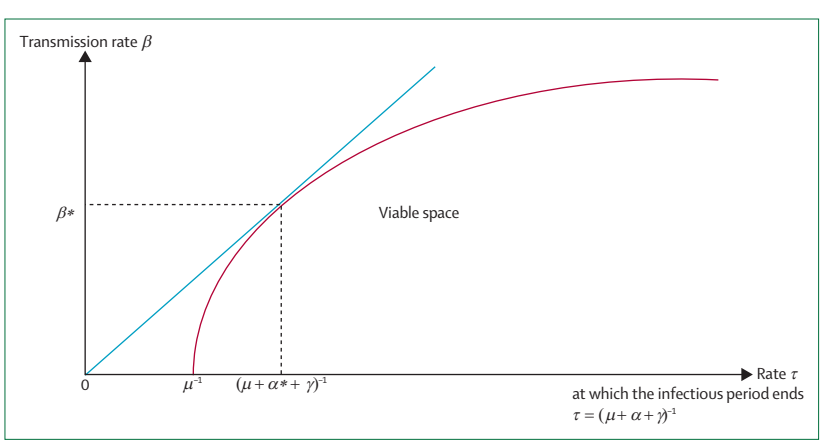

Figure 1: **AVS illustrated in a simple parasitic lifecycle**
Consider a parasite spreading by contact within a single host population and two of these genetically coded traits: transmission rate (β) and virulence (α) referring to host mortality induced by infection. Parasite fitness per unit of time is given by $R = \beta / (m+\alpha+g)$, where m is the host natural mortality and g is the time of parasite clearance. The red curve describes the transmission-virulence trade-off (ie, the biological constraints describing the links between virulence, transmission, and fitness) among extant parasite genotypes. The viable space bounded by the trade-off curve and the x-axis determines the array of parasite strategies (genotypes) allowing the persistence of both the species. Outbreaks starting within the viable space will be followed by selection favouring mutants with higher transmission and longer infection periods until the trade-off curve is reached. The strategy defined by $\alpha=\alpha^*$ and $\beta=\beta^*$ is that conferring the maximum possible fitness for the parasite. Directly investigating variations in parasite fitness with virulence rather than the trade-off curve facilitates empirical tests for the AVS.[8] AVS=adaptive virulence scenario.

The CVS posits an increased likelihood of epidemiological networks including the circulation of several pathogens across several species, such that several pathogens co-infect some individuals and a given pathogen infects several species. A comprehensive understanding of infection dynamics in these epidemiological networks can lead to innovative strategies in disease prevention and management.[18] Disease ecologists study how heterogeneities in space and time among individuals and populations (including the effects of co-infection), host species (interacting differently with pathogens), and regions (varying in species assemblages) influence infection dynamics in these systems.[18] Disease ecologists reach this goal by assessing the relative contributions of susceptible species to pathogen persistence in space, identifying reservoirs and bridge hosts. The presence of a reservoir is enough to ensure the persistence of a pathogen population. Bridge hosts, often involved in the transmission of zoonotic pathogens to humans, are also susceptible to infection and competent for pathogen transmission but greatly vary in their competence for pathogen replication.

We argue that the historical focus only on mycobacteria-vertebrate interactions could have overlooked the possibilities of diseases involving the CVS. This argument is supported by the extant literature on mycobacteria, which uses search methodologies designed for testing the alternative evolutionary hypotheses for the origin of the selection for mycobacterial pathogenicity (appendix p 1). We finally discuss the intellectual and translation gains that could result from investigating the phylogenetic relationships among pathogenic and non-pathogenic mycobacteria. Mycobacteria, including many NTMs yet to be studied, occur in soil and aquatic habitats.[19,20] Assessing the relatedness and similarity of traits of these mycobacteria with known pathogens can provide insights for predicting new and emerging infectious diseases associated with current global changes.

Vertebrate-driven selection in transmissible mycobacteria

Three *M abscessus* genotypes have been identified as indirectly transmitted human parasites in human-engineered habitats.[21] The recurrent mutations observed in the *PhoR* gene and at the glycopeptidolipid locus in these genotypes affect their survival in the environment,[21] which correlates with the tenets of the AVS.

The clonal lineages within MTBC arose as hominid parasites circa 2·8 million years ago.[22] Phylogenomic studies have revealed co-diversification of L1–L9 lineages with some human populations and diversification of zoonotic MTBC

clones from hominid-infecting bacteria related to L5–L6.[23–26] Clinical surveys provide data supporting the adequacy of the AVS to the evolution of MTBC in humans.[27] Bacilli replacements from older clones (L1, L5, and L6) with L2–L4 modern clones arose with different immunopathology symptoms and increased transmission.[27] L2–L4 cocirculation in multiethnic populations was also informative.[27] In such cases, the likelihood of experiencing tuberculosis symptoms (necessary for transmission) increases when infection occurs in humans with similar genetic backgrounds rather than when it occurs in individuals with whom their ancestors have coevolved (ie, different Asian populations for L2–L3 and Europeans for L4); however, this pattern is not seen in patients with increased disease risk (eg, those with HIV co-infection).[27] Since, on average, only 10% of human-infecting bacilli complete their lifecycle,[27] MTBC evolving in distinct human populations most likely optimised their fitness by shaping adaptations specific to human genetic backgrounds. Regarding zoonotic MTBC clones, the vertebrate-driven patterns observed in phylogenies also support the AVS: their pathogenicity most likely results from specific adaptations to distinct animals.[26] However, multihost epidemiological networks have been studied in *Mycobacterium bovis*, adapted to taurine cattle (*Bos taurus*).[26] If humans are dead-end hosts for *M bovis*, diverse animals (eg, deer, ferrets, and boars) are bridge hosts, whereas the brushtail possum (*Trichosurus vulpecula*) and European badger (*Meles meles*) are efficient reservoirs.[28,29]

Mycobacterium avium subspecies *paratuberculosis* (MAP) was described as a parasite alternating between soil-dwelling free-living stages and livestock intestinal cell infections. In early studies, host-specific adaptations to either cattle or sheep were suspected because genetic differentiation and pathogenicity differences among MAP strains were related to their vertebrate origin.[30] However, phylogenomic studies challenged this view.[30,31] The diversity of wildlife suspected of playing an epidemiological role, as bridge hosts or reservoirs, increased with studies documenting wildlife species associated with MAP (see a selected reference list in appendix pp 1–2). In particular, the European rabbit (*Oryctolagus cuniculus*) emerged as a reservoir affecting infection exposure in cattle.[32,33] Furthermore, MAP epidemiology in Portuguese wild carnivores does not depend on MAP infections in cattle.[34] Monitoring MAP abundance in diverse habitats (eg, sediments, rivers, and lakes) along a catchment basin from livestock sources to drinking water found that all aquatic habitats contributed to MAP persistence.[35] The authors opined that some amoebae (*Acanthamoeba* spp) could be efficient reservoirs. Assessing MAP carriage in soil-dwelling amoebae also suggested alternatives to the lifecycle of MAP that do not include vertebrate infection, with reported identical exposure to infection for samples from pastures routinely grazed by cattle or crop fields that had no contact with livestock and manure for 10 to 40 years.[36] The overall pattern is more in agreement with the CVS than with the AVS. The case of leprosy bacilli is similar to that of MAP.

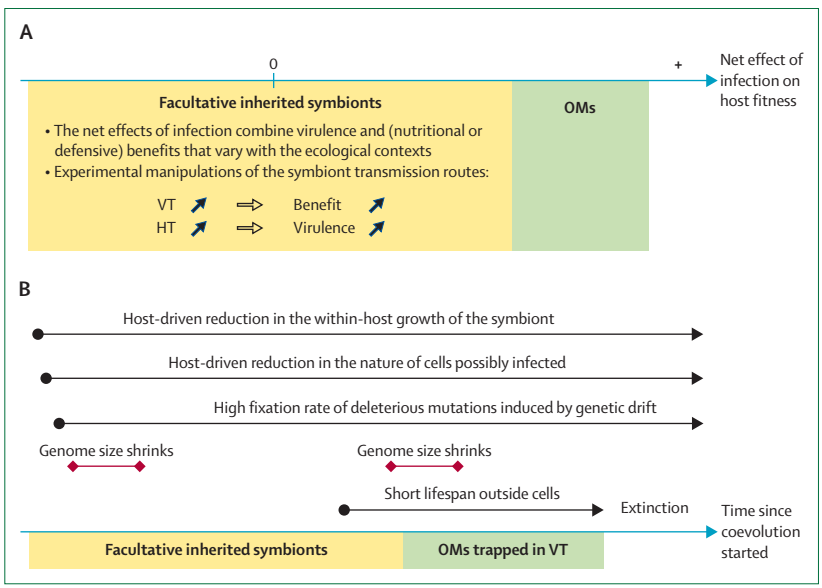

Figure 2: **Vertical transmission: symbiont adaptation under maximum host control**
(A) The sources of variations in the effects of infection on host fitness, which make them shift recurrently between negative and positive values in time and space. When the average of the net infection effect across the landscape is positive, hosts should maximise VT and reduce symbiont virulence. (B) The hosts do both,[9] which has striking cascading consequences for the inherited symbiont, including a drastic reduction in symbiont population sizes (enhancing effects of mutation bias and genetic drift) and limitation of recombination opportunities that help to get rid of the accumulating deleterious mutations. Functional metabolic pathways thus decrease in number over time, even when already restricted to those allowing intracellular life and providing benefits to the host. Under such circumstances, symbionts evolving under strict VT are driven to extinction. Phylogenomic studies performed on inherited symbionts associated with insects confirmed these patterns and revealed two main events of marked reductions in genome sizes along the way.[9–11] Thus, symbiont genomes are longer in early coevolutionary stages than in late coevolutionary stages but bear a higher pseudogene content in early coevolutionary stages than in late coevolutionary stages.[10,11] HT=horizontal transmission. OM=obligate mutualists. VT=vertical transmission.

Leprosy bacilli: multihost systems within ecosystems

Humans were long thought to be unique leprosy reservoirs, with approximately 5% of infected people developing leprosy that affects skin dermis, peripheral nerves, and mucosa of the upper respiratory tract.[37] Transmission, thought to be mediated by coughing and nasal excreta of symptomatic individuals, requires close and repeated contacts.[37] However, reports of *M leprae* spillovers exist in diverse wild animals with which humans are unlikely to live in close contact.[38] Furthermore, *M leprae* circulates in wildlife, including in Eurasian red squirrels (*Sciurus vulgaris*) in the British Isles, in wild armadillos in the Americas, and in west African wild chimpanzees, with remote populations experiencing outbreaks.[39–41] Phylogenomic analyses provided evidence of epidemiological continuity among neighbouring human and animal strains, ruling out hypotheses of vertebrate-tropism difference and vertebrate-driven genetic divergence.[40] Epidemics in west African apes suggest the role of unknown intermediate (animal or environmental) reservoirs as the strains involved have been detected at a low frequency in nearby human populations.[41] Although the survival of *M leprae* outside of cells is short, this bacterium is present in soil and

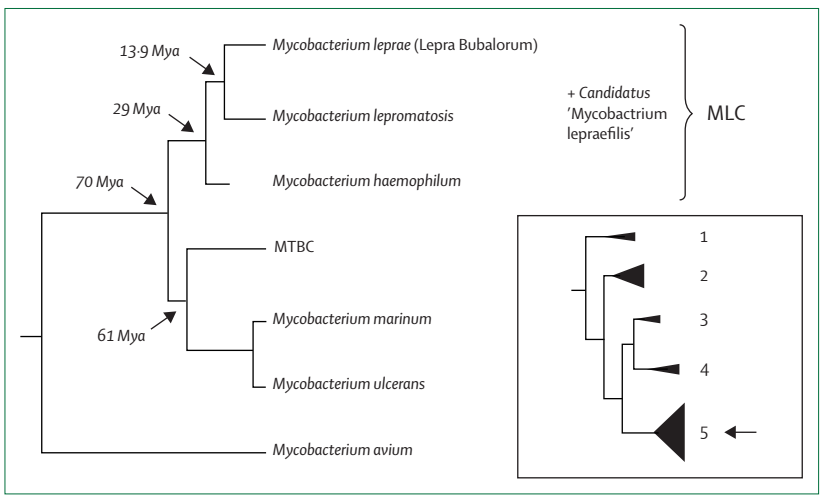

Figure 3: **MLC and divergence estimates among major human pathogens[42]**
Lepra Bubalorum is a mycobacterial infection in Indonesian water buffaloes assumed to be caused by *Mycobacterium leprae*.[43] Aside from a close relationship with *M leprae* and *Mycobacterium lepromatosis*, the exact phylogenetic position of *Candidatus* 'Mycobacterium lepraefelis' relative to that of *Mycobacterium haemophilum* remains unknown. The inset replaces the cladogram within the mycobacterial phylogeny.[44] The correspondence between the main clades and labels 1–5 is as follows. 1: abscessus-chelonae. 2: fortuitum-vacae. 3: triviale. 4: terrae. 5: tuberculosis-simiae. MLC=*Mycobacterium leprae* complex. MTBC=*Mycobacterium tuberculosis* complex. Mya=million years ago.

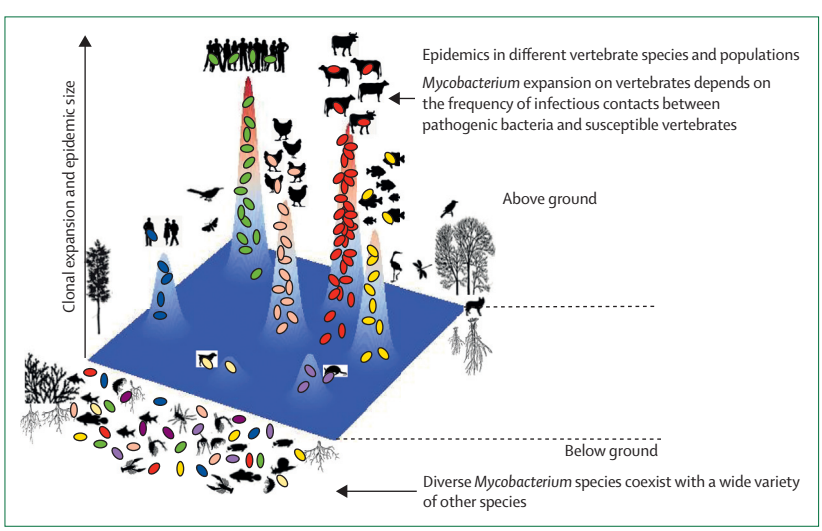

Figure 4: **Biodiversity ensures a high diversity of *Mycobacterium* species and circumstantial situations, including high animal or human abundance and density, to facilitate the expansion of the bacillus strain**
Illustration of *Mycobacterium* species diversity, represented by the coloured ovals in natural ecosystems and epidemic outbreaks. Diverse *Mycobacterium* species coexist with the several competitors, predators, and host or non-host species (ie, below-ground profile) in species-rich ecosystems. Epidemic outbreaks with strains of mycobacteria, and even clonal expansion of the same strain, could happen when one population of a given vertebrate species is exposed to a sufficient number and density of mycobacterial pathogens (ie, above-ground profile). The figure illustrates different disease epidemics for human populations, poultry, cattle farms, and fish aquaculture as breeding situations in the background. The extent of an epidemic corresponds to the epidemic population size. In the foreground, small outbreaks are shown, which are representative of atypical mycobacteria. Below-ground and above-ground profiles correspond to two distinct faces of the same coin. High species and genetic diversity in mycobacteria bacilli in natural ecosystems, as studied by environmental microbiologists, ecologists, and evolutionary biologists, might circumstantially generate epidemic outbreaks in animal or human populations, as analysed by veterinarians and medical doctors, depending on the host demography and environmental stochasticity.

water and remains viable and transmissible after being hosted by amoebae.[38]

The closest relatives of *M leprae* share several traits and a study by Ploemacher and colleagues suggests a need for regrouping them within the *M leprae* complex (MLC; figure 3).[38] MLC includes *Mycobacterium lepromatosis* that infects humans in several countries (eg, Mexico, Canada, Brazil, Myanmar, and Singapore).[42] *M lepromatosis* and *M leprae* promote similar disease symptoms, present similar genomic organisation (eg, massive gene decay and small genome size relative to other mycobacteria), and share mammalian hosts.[42] *M lepromatosis* also circulates among Eurasian red squirrels in the British Isles, with mycobacterial divergence from a common ancestor of human-infecting Mexican strain about 27 000 years ago.[39] Another MLC member, *Candidatus* 'Mycobacterium lepraefelis', is an agent of feline leprosy,[45] suggesting that MLC members can share similar virulence factors.

Overall, there is a low probability that a vertebrate might have been a reservoir for *M lepromatosis* and *M leprae*. As per an alternative hypothesis, vertebrate infections would rely on spillover from non-vertebrate reservoirs, which is more suitable for explaining the persistence of *M leprae* infections despite the poor efficiency of *M leprae* in interhuman transmission.[40] *M leprae* and *M lepromatosis* share several traits (ie, slow growth rate, short life span outside of cells, and reduced genome sizes with high pseudogene content in comparison with that in their relatives)[42] with inherited symbionts (figure 2B). We suggest that adopting research approaches used for other NTMs, such as *M ulcerans*, could help to better understand and explain the environmental existence of MLC species and their pathogenicity origins.

Identifying the ecological drivers in other diseases: the *M ulcerans* example

Studies by environmental microbiologists and ecologists can provide useful information on the factors affecting NTM exposure through time and space (figure 4). Such data can also shed light on possible multiple functions of NTM virulence factors. The case of *M ulcerans*, the etiological agent of Buruli ulcer, is an interesting example.

M ulcerans is one of the mycolactone-producing mycobacteria.[46] Mycolactone-producing mycobacteria arose from *Mycobacterium marinum* chromosomal backgrounds and upon acquisition of a giant plasmid responsible for production of the cytotoxic mycolactone lipid that causes disease in humans and animals.[46] A comprehensive review of field studies over the past 50 years (1971–2020) showed that adopting an ecological and evolutionary framework is essential for deciphering the multiple potential transmission routes of *M ulcerans*.[47] A wide diversity of organisms can carry *M ulcerans*, including terrestrial vertebrates (eg, possum in Australia and the rodent grasscutter in west Africa), aquatic organisms (eg, plant, fish, insect, annelid, and mollusc), and terrestrial arthropods (eg, caterpillar and spider).[47] These organisms are preferably called (host) carriers until the exact contribution of each

organism to the environmental persistence of *M ulcerans* is identified. The best strategy for identifying the drivers of environmental persistence of *M ulcerans* is to build a dataset based on two principles: an equal consideration of all possible carriers through space and time and systematic screening of possible carriers for the presence of *M ulcerans*. This approach allows for an unbiased assessment of the biotic and abiotic factors that influence the environmental abundance of the pathogen.[47] Ecological perturbations, landscape change, deforestation, seasonal variations in rainfall, fluctuations in both abiotic factors (eg, pH, dissolved oxygen, flow speed) and biotic factors (diversity and abundance of the species in a given water body) affect *M ulcerans* loads across a large diversity of carriers.[47] Moreover, considering the pathogen prevalence across all carriers, rather than only in potential or suspected vectors, was shown as a prerequisite for correctly evaluating human exposure to *M ulcerans* infection.[48]

When *M ulcerans* undergoes local extinction, its carriage by aquatic arthropods and organic matter appears as the most likely explanation for recolonisation in a water body, constituting the earliest indicator of human disease risks.[49] Laboratory experiments provided functional explanations for these field observations, as higher chitin concentration in the culture medium increased growth and pH tolerance in *M ulcerans*.[50,51] Production of mycolactone, a chemoattractant for some fungi,[52] could favour contacts with chitinous compounds (present in fungi cell walls) affecting *M ulcerans* metabolism. Culturing *M ulcerans* under optimal nutritional conditions resulted in lower mycolactone production, cells stopping toxin production despite bearing the plasmid, or selecting cells that lost the plasmid.[53–55] This signal convergence across laboratories and *M ulcerans* strains indicates most likely increases in fitness advantages provided by the toxin to *M ulcerans* in nutrient-poor environments because of other pleiotropic roles of mycolactone (eg, conferring a competitive or prey-predator advantage). Thus, exposure to *M ulcerans* infection most likely fluctuates across seasons. Highly abundant *M ulcerans* populations with low toxicity would be associated with greater diversity and abundance of potential carriers (eg, arthropods and freshwater fish blooms) in rainy seasons. *M ulcerans* would be less abundant but more toxic in late dry seasons, when favourable habitats become scarcer, and both environmental and climatic conditions become more drastic for bacterial survival. Research on other pathogenic NTMs could use a similar approach for addressing pathogen maintenance in space and time and pathogenicity variations across environmental contexts.

A similar scenario in which NTM pathogenicity and abundance fluctuate with the densities of diverse carriers could partly explain why fish mycobacterial epidemics coincide with ecological perturbations.[56] The existing consensus from fish aquaculture research suggests that contamination usually starts with the introduction of NTM-infected prey (eg, the ciliate *Paramecium caudatum*, brine shrimp *Artemia franciscana*, and rotifers *Branchionus* spp) to a fish population.[57,58] NTM maintenance then relies on fish acquisition through ingestion of mycobacterial biofilms, NTM release in fish faeces, and fish-to-fish transmission through cannibalism or wounds.[57,58] The fact that cocultivation of *M marinum* and *M chelonae* with *P caudatum* increases transmission and pathogenicity in some fish specimens could indicate the predominance of ciliates over alternative non-vertebrates as infectious sources.[59] However, this point requires further confirmation.

The diversity of organisms involved in virulence and environmental persistence remains unresolved for most NTMs. The divergence patterns between pathogenic and non-pathogenic NTMs might provide some information on whether similarities could be expected among NTMs.

Evolution of pathogenicity in mycobacteria: consequences for disease ecology and medicine

The number of valid mycobacterial species has increased drastically with advances in genomic technologies and the increased ability of the scientific community to evaluate environmental samples.[1] Ranging from 10 to 61 between

	Clade name (proposed new or amended genus names)	N_{tot}	Major human pathogens	Fish pathogens[57]
1	abscessus-chelonae (*Mycobacteroides*)	≥7	*Mycobacterium abscessus* (subsp *abscessus, bolleti*) *Mycobacterium chelonae* *Mycobacterium frankilini* *Mycobacterium immunogenum*	*M abscessus* *M chelonae* *Mycobacterium salmonophilum*
2	fortuitum-vaccae (*Mycolicibacterium*)	≥49	*Mycobacterium fortuitum*	*Mycobacterium flavescens* *M fortuitum* *Mycobacterium holsaticum* *Mycobacterium neoaurum* *Mycobacterium peregrinum* *Mycobacterium septicum* *Mycobacterium smegmatis*
3	triviale (*Mycolicibacillus*)	≥5	*Mycobacterium triviale*	*M triviale*
4	terrae (*Mycolicibacter*)	≥14	*Mycobacterium terrae*	*M terrae*
5	tuberculosis-simiae (emended *Mycobacterium* genus)	≥83	MAC (subsp *avium, intracellulare*) *Mycobacterium gordonae* MLC (*Mycobacterium leprae, Mycobacterium lepromatosis*) MKC (*Mycobacterium kansaii*) *Mycobacterium marinum-ulcerans* *Mycobacterium scrofulaceum* *Mycobacterium paragordonae* *Mycobacterium szulgai* MTBC *Mycobacterium xenopi*	MAC (subsp *avium, intracellulare*) *M gordonae* MLC (*Mycobacterium haemophilum*) *Mycobacterium lentiflavum* *M marinum-ulcerans* *Mycobacterium simiae*

The first two columns refer to the deepest divergence events observed along the mycobacteria phylogeny: order number in the first column=order of diversification (ie, numbers used in figure 3 inset) and clade name in the second column=current double denomination of the five main clades. The classic names given to the main clades are followed by, in parentheses, those given in a recently proposed systematic revision. [44] Specialists of mycobacteria evolution argue against this revision.[61] This approach limits the number of taxa that unambiguously appear as belonging to either one of the five main clades in the List of Prokaryotic names with Standing in Nomenclature database,[60] and thus, this number (N_{tot}) is a minimal estimate. Taxa are listed in alphabetical order of name to emphasise the similarity between the lists of major human and fish pathogens. MAC=*M avium* complex. MLC=*M leprae* complex. MKC=*M kansaii* complex. MTBC=*M tuberculosis* complex.

Table: **Distribution of major human and fish mycobacterial pathogens among the main clades structuring the mycobacterial phylogeny**

the 1960s and 2010s,[1] this number was 196 in 2023, according to the List of Prokaryotic Names with Standing in Nomenclature database.[60] This trend will most likely continue, given the results of a global and systematic survey of soil-dwelling mycobacteria using two loci.[20] Indeed, only five (3%) of the 159 discriminated lineages in that study were present in genetic databases.[20]

Genomic analyses revealed the coexistence of non-pathogenic and pathogenic taxa within each of the main mycobacterial clades (table), a pattern that has also been observed within the *M avium* and *M kansasii* complexes.[44,62,63] The acquisition (or loss, or both) of pathogenicity, thus, occurred many times during mycobacterial evolution.[64] Estimates of mycobacterial divergence times are too few to properly assess the timings of pathogenicity. The information available only indicates that diverse representatives of the main clades existed 70 million years ago (figure 3). A global survey of soil-dwelling mycobacteria nonetheless revealed substantial constraints (eg, vegetation cover, climatic zone, and soil pH) that shaped the distribution of 159 mycobacterial lineages across 143 soil samples.[20] This finding suggests that mycobacteria most likely adapted to different conditions along their specific evolution, including differences in interspecific interactions with competitors or predators or potential hosts, or a combination of these.

Research on human-engineered habitats provides evidence that mycobacteria have physiological traits favouring their colonisation of plumbing systems and spillover to humans.[19] This process is facilitated by the suitability of plumbing for amoebae, since pathogenic mycobacteria (except *M fortuitum*) can survive in amoebae.[65,66] Some studies suggest that mycobacteria pathogenicity arose in the amoeba,[67,68] on the basis of the finding that in cases in which the molecular basis of amoeba and macrophage infections were identified, both traits depended on the same locus (*ESX-1* in MTBC, *M marinum*, and *M kansasii*; *ESX-4* in *M abscessus;* one pathogenicity island in *M avium* complex).[68–70] However, distinguishing cases of amoeba-NTM coevolution (leading to NTM persistence in amoeba populations through horizontal or vertical transmission) from those in which defensive and infective functions have coincidentally recruited the same genes is not enough. Amoeba-NTM coevolution could be relevant only for NTMs that replicate in amoeba cells, such as those reported in *M kansasii* and *M avium* complexes.[66] The fact that *M kansasii* recombinant clones emerged with the use of plumbing networks, and the evidence of positive selection at the *ESX-1* locus, supports the hypothesis of a selective role for amoebae in pathogenicity.[63] Some data are also consistent with the hypothesis of coevolution between *M avium* complex and *Acanthamoeba* species. Coculturing *M avium* and *Acanthamoeba castellanii* for 10 days enhanced pathogenicity in mice and infectivity and growth in human macrophages.[71] Replicating the experiment using the same bacterial strain and *Acanthamoeba lenticulata* provided similar results after 12 days, but not after 42 days, at which point there were reductions in replication within amoeba and persistence in human macrophages.[72] Further, other strains of the *M avium* complex exploit *Acanthamoeba* species.[65,66,73] By contrast, *M chelonae* and *M marinum* infect and survive in amoeba cells but multiply in a ciliate, which then enhances their virulence and transmission to zebrafish.[59] For these NTMs, as for others not replicating in amoeba cells, the amoebae colonising plumbing systems are more likely to represent bridge hosts than the selective origin of pathogenicity.

Conclusions and perspectives

Mycobacteria that have coevolved with vertebrates through a specific parasitic lifecycle are rare (restricted to MTBC and three *M abscessus* genotypes so far). Mycobacteria have a long history of occupying diverse habitats in which their phenotypic and genetic traits (including pathogenicity) evolved with conditions unrelated to vertebrates. Therefore, information acquired solely from patients, diseased animals, and human-engineered habitats hinders the identification of putative non-vertebrate hosts and environmental reservoirs that are important in the development of novel approaches for the prevention of disease. Overall, the environmental life of pathogenic mycobacteria requires many additional scientific inquiries that adopt ecological and evolutionary perspectives of the disease for deciphering cases wherein pathogenicity arose as a coincidental consequence of evolution in complex and species-rich natural environments. This approach is becoming increasingly important since mycobacteria are projected to become more abundant and distributed under global scenarios of climate warming, soil acidification, and land use associated with soil erosion and agriculture development.[74]

Contributors

CC, J-FG, and MEB conceptualised the paper, performed most of the literature search, and wrote the first draft. CC and J-FG produced the figures. BdT, AWR, KMF, JLP, SP, LE, PLT, MD, HRJ, and MWS contributed to previous and final drafts through substantial additions, critical review, and commentary.

Declaration of interests

We declare no competing interests. The content of the article is the sole responsibility of the authors, with no role played by the funding agencies.

Acknowledgments

This work was supported by the joint NSF-NIH-NIFA Ecology and Evolution of Infectious Disease program (DEB grant number 1911457). CC, BdT, and J-FG benefited from an Investissement d'Avenir grant managed by Agence Nationale de la Recherche (LABEX CEBA: ANR-10-LABX-25-01). CC was supported by Institut de Recherche pour le Développement (IRD), Centre National pour la Recherche Scientifique, and Université de Montpellier (UM). J-FG was supported by IRD, UM, Institut national de Recherches pour l'Agriculture, l'Alimentation et l'Environnement, and Ecole des Hautes Etudes en Santé Publique. MEB received an award from an Investissement d'Avenir grant managed by Agence Nationale de la Recherche (LABEX CEMEB: ANR-10-LABX-04-01) and support by Michigan State Agbioresearch, College of Agriculture and Natural Resources, Department of Entomology, and Department of Osteopathic Medical Specialties. PLT benefited from a grant managed by Agence Nationale de la Recherche sur le sida et les hépatites virales/Maladies Infectieuses Emergentes (ANRS MIE).

References

1 Turenne CY. Nontuberculous mycobacteria: insights on taxonomy and evolution. *Infect Genet Evol* 2019; **72:** 159–68.

2 WHO. Global tuberculosis report 2022. Geneva: World Health Organization; Oct 27, 2022. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022 (accessed Sept 2, 2023).

3 WHO. Towards zero leprosy. Global leprosy (Hansen's disease) strategy 2021–2030. New Delhi, India: WHO Regional Office for South-East Asia; April 15, 2021. https://www.who.int/publications/i/item/9789290228509 (accessed Sept 2, 2023).

4 Simpson H, Deribe K, Tabah EN, et al. Mapping the global distribution of Buruli ulcer: a systematic review with evidence consensus. *Lancet Glob Health* 2019; **7:** e912–22.

5 Prevots DR, Loddenkemper R, Sotgiu G, Migliori GB. Nontuberculous mycobacterial pulmonary disease: an increasing burden with substantial costs. *Eur Respir J* 2017; **49:** 1700374.

6 Hoefsloot W, van Ingen J, Andrejak C, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. *Eur Respir J* 2013; **42:** 1604–13.

7 Alizon S, Hurford A, Mideo N, Van Baalen M. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J Evol Biol* 2009; **22:** 245–59.

8 Alizon S, Michalakis Y. Adaptive virulence evolution: the good old fitness-based approach. *Trends Ecol Evol* 2015; **30:** 248–54.

9 Drew GC, Stevens EJ, King KC. Microbial evolution and transitions along the parasite-mutualist continuum. *Nat Rev Microbiol* 2021; **19:** 623–38.

10 Lo W-S, Huang Y-Y, Kuo C-H. Winding paths to simplicity: genome evolution in facultative insect symbionts. *FEMS Microbiol Rev* 2016; **40:** 855–74.

11 Latorre A, Manzano-Marín A. Dissecting genome reduction and trait loss in insect endosymbionts. *Ann N Y Acad Sci* 2017; **1389:** 52–75.

12 Weinert LA, Werren JH, Aebi A, Stone GN, Jiggins FM. Evolution and diversity of *Rickettsia* bacteria. *BMC Biol* 2009; **7:** 6.

13 Levin BR, Svanborg Edén C. Selection and evolution of virulence in bacteria: an ecumenical excursion and modest suggestion. *Parasitology* 1990; **100** (suppl): S103–15.

14 Rivera-Pérez JI, González AA, Toranzos GA. From evolutionary advantage to disease agents: forensic reevaluation of host-microbe interactions and pathogenicity. *Microbiol Spectr* 2017; **5**. https://doi.org/10.1128/microbiolspec.EMF-0009-2016.

15 Adiba S, Nizak C, van Baalen M, Denamur E, Depaulis F. From grazing resistance to pathogenesis: the coincidental evolution of virulence factors. *PLoS One* 2010; **5:** e11882.

16 Brown SP, Cornforth DM, Mideo N. Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. *Trends Microbiol* 2012; **20:** 336–42.

17 Kapitein N, Mogk A. Deadly syringes: type VI secretion system activities in pathogenicity and interbacterial competition. *Curr Opin Microbiol* 2013; **16:** 52–58.

18 Johnson PT, de Roode JC, Fenton A. Why infectious disease research needs community ecology. *Science* 2015; **349:** 1259504.

19 Falkinham JO IIIrd. Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *J Appl Microbiol* 2009; **107:** 356–67.

20 Walsh CM, Gebert MJ, Delgado-Baquerizo M, Maestre FT, Fierer NA. A global survey of mycobacterial diversity in soil. *Appl Environ Microbiol* 2019; **85:** e01180-19.

21 Bryant JM, Brown KP, Burbaud S, et al. Stepwise pathogenic evolution of *Mycobacterium abscessus*. *Science* 2021; **372:** eabb8699.

22 Brisse S, Supply P, Brosch R, Vincent V, Gutierrez MC. "A reevaluation of *M. prototuberculosis*": continuing the debate. *PLoS Pathog* 2006; **2:** e95.

23 Comas I, Coscolla M, Luo T, et al. Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. *Nat Genet* 2013; **45:** 1176–82.

24 Ngabonziza JCS, Loiseau C, Marceau M, et al. A sister lineage of the *Mycobacterium tuberculosis* complex discovered in the African Great Lakes region. *Nat Commun* 2020; **11:** 2917.

25 Coscolla M, Gagneux S, Menardo F, et al. Phylogenomics of *Mycobacterium africanum* reveals a new lineage and a complex evolutionary history. *Microb Genom* 2021; **7:** 000477.

26 Brites D, Loiseau C, Menardo F, et al. A new phylogenetic framework for the animal-adapted *Mycobacterium tuberculosis* complex. *Front Microbiol* 2018; **9:** 2820.

27 Gagneux S. Host-pathogen coevolution in human tuberculosis. *Philos Trans R Soc Lond B Biol Sci* 2012; **367:** 850–59.

28 Allen AR. One bacillus to rule them all? – investigating broad range host adaptation in *Mycobacterium bovis*. *Infect Genet Evol* 2017; **53:** 68–76.

29 Nugent G. Maintenance, spillover and spillback transmission of bovine tuberculosis in multi-host wildlife complexes: a New Zealand case study. *Vet Microbiol* 2011; **151:** 34–42.

30 Bryant JM, Thibault VC, Smith DG, et al. Phylogenomic exploration of the relationships between strains of *Mycobacterium avium* subspecies *paratuberculosis*. *BMC Genomics* 2016; **17:** 79.

31 Hodgeman R, Mann R, Savin K, Djitro N, Rochfort S, Rodoni B. Molecular characterisation of *Mycobacterium avium* subsp. *paratuberculosis* in Australia. *BMC Microbiol* 2021; **21:** 101.

32 Judge J, Kyriazakis I, Greig A, Allcroft DJ, Hutchings MR. Clustering of *Mycobacterium avium* subsp. *paratuberculosis* in rabbits and the environment: how hot is a hot spot? *Appl Environ Microbiol* 2005; **71:** 6033–38.

33 Fox NJ, Caldow GL, Liebeschuetz H, Stevenson K, Hutchings MR. Counterintuitive increase in observed *Mycobacterium avium* subspecies *paratuberculosis* prevalence in sympatric rabbits following the introduction of paratuberculosis control measures in cattle. *Vet Rec* 2018; **182:** 634.

34 Cunha MV, Rosalino LM, Leão C, et al. Ecological drivers of *Mycobacterium avium* subsp. *paratuberculosis* detection in mongoose (*Herpestes ichneumon*) using IS900 as proxy. *Sci Rep* 2020; **10:** 860.

35 Pickup RW, Rhodes G, Bull TJ, et al. *Mycobacterium avium* subsp. *paratuberculosis* in lake catchments, in river water abstracted for domestic use, and in effluent from domestic sewage treatment works: diverse opportunities for environmental cycling and human exposure. *Appl Environ Microbiol* 2006; **72:** 4067–77.

36 White CI, Birtles RJ, Wigley P, Jones PH. *Mycobacterium avium* subspecies *paratuberculosis* in free-living amoebae isolated from fields not used for grazing. *Vet Rec* 2010; **166:** 401–02.

37 Suzuki K, Akama T, Kawashima A, Yoshihara A, Yotsu RR, Ishii N. Current status of leprosy: epidemiology, basic science and clinical perspectives. *J Dermatol* 2012; **39:** 121–29.

38 Ploemacher T, Faber WR, Menke H, Rutten V, Pieters T. Reservoirs and transmission routes of leprosy; a systematic review. *PLoS Negl Trop Dis* 2020; **14:** e0008276.

39 Avanzi C, Del-Pozo J, Benjak A, et al. Red squirrels in the British Isles are infected with leprosy bacilli. *Science* 2016; **354:** 744–47.

40 Avanzi C, Singh P, Truman RW, Suffys PN. Molecular epidemiology of leprosy: an update. *Infect Genet Evol* 2020; **86:** 104581.

41 Hockings KJ, Mubemba B, Avanzi C, et al. Leprosy in wild chimpanzees. *Nature* 2021; **598:** 652–56.

42 Silva FJ, Santos-Garcia D, Zheng X, Zhang L, Han XY. Construction and analysis of the complete genome sequence of leprosy agent *Mycobacterium lepromatosis*. *Microbiol Spectr* 2022; **10:** e0169221.

43 Faber WR, Menke H, Rutten V, Pieters T. Lepra bubalorum, a potential reservoir of *Mycobacterium leprae*. *Front Microbiol* 2021; **12:** 786921.

44 Gupta RS, Lo B, Son J. Phylogenomics and comparative genomic studies robustly support division of the genus *Mycobacterium* into an emended genus *Mycobacterium* and four novel genera. *Front Microbiol* 2018; **9:** 67.

45 O'Brien CR, Malik R, Globan M, Reppas G, McCowan C, Fyfe JA. Feline leprosy due to *Candidatus* 'Mycobacterium lepraefelis': further clinical and molecular characterisation of eight previously reported cases and an additional 30 cases. *J Feline Med Surg* 2017; **19:** 919–32.

46 Pidot SJ, Asiedu K, Käser M, Fyfe JA, Stinear TP. *Mycobacterium ulcerans* and other mycolactone-producing mycobacteria should be considered a single species. *PLoS Negl Trop Dis* 2010; **4:** e663.

47 Receveur JP, Bauer A, Pechal JL, et al. A need for null models in understanding disease transmission: the example of *Mycobacterium ulcerans* (Buruli ulcer disease). *FEMS Microbiol Rev* 2022; **46:** fuab045.

48 Garchitorena A, Ngonghala CN, Texier G, et al. Environmental transmission of *Mycobacterium ulcerans* drives dynamics of Buruli ulcer in endemic regions of Cameroon. *Sci Rep* 2015; **5:** 18055.

49 García-Peña GE, Garchitorena A, Carolan K, et al. Niche-based host extinction increases prevalence of an environmentally acquired pathogen. *Oikos* 2016; **125:** 1508–15.

50 Sanhueza D, Chevillon C, Colwell R, et al. Chitin promotes *Mycobacterium ulcerans* growth. *FEMS Microbiol Ecol* 2016; **92:** fiw067.

51 Sanhueza D, Chevillon C, Boumzibi N, Godreuil S, Guégan J-F. Chitin increases *Mycobacterium ulcerans* growth in acidic environment. *Microbes Environ* 2018; **2:** 234–37.

52 Hammoudi N, Cassagne C, Armstrong N, et al. *Mycobacterium ulcerans* mycolactones-fungi crosstalking. *Sci Rep* 2019; **9:** 3028.

53 Deshayes C, Angala SK, Marion E, et al. Regulation of mycolactone, the *Mycobacterium ulcerans* toxin, depends on nutrient source. *PLoS Negl Trop Dis* 2013; **7:** e2502.

54 Sanhueza D, Guégan J-F, Jordan H, Chevillon C. Environmental variations in *Mycobacterium ulcerans* transcriptome: absence of mycolactone expression in suboptimal environments. *Toxins* 2019; **11:** 146.

55 Nakanaga K, Ogura Y, Toyoda A, et al. Naturally occurring a loss of a giant plasmid from *Mycobacterium ulcerans* subsp. *shinshuense* makes it non-pathogenic. *Sci Rep* 2018; **8:** 8218.

56 Kane AS, Stine CB, Hungerford L, Matsche M, Driscoll C, Baya AM. Mycobacteria as environmental portent in Chesapeake Bay fish species. *Emerg Infect Dis* 2007; **13:** 329–31.

57 Delghandi MR, El-Matbouli M, Menanteau-Ledouble S. Mycobacteriosis and infections with non-tuberculous mycobacteria in aquatic organisms: a review. *Microorganisms* 2020; **8:** 1368.

58 Chang CT, Lewis J, Whipps CM. Source or sink: examining the role of biofilms in transmission of *Mycobacterium* spp. in laboratory zebrafish. *Zebrafish* 2019; **16:** 197–206.

59 Peterson TS, Ferguson JA, Watral VG, Mutoji KN, Ennis DG, Kent ML. *Paramecium caudatum* enhances transmission and infectivity of *Mycobacterium marinum* and *M. chelonae* in zebrafish *Danio rerio*. *Dis Aquat Organ* 2013; **106:** 229–39.

60 Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol* 2020; **70:** 5607–12.

61 Tortoli E, Brown-Elliott BA, Chalmers JD, et al. Same meat, different gravy: ignore the new names of mycobacteria. *Eur Respir J* 2019; **54:** 1900795.

62 Ignatov D, Kondratieva E, Azhikina T, Apt A. *Mycobacterium avium*-triggered diseases: pathogenomics. *Cell Microbiol* 2012; **14:** 808–18.

63 Luo T, Xu P, Zhang Y, et al. Population genomics provides insights into the evolution and adaptation to humans of the waterborne pathogen *Mycobacterium kansasii*. *Nat Commun* 2021; **12:** 2491.

64 Leclerc MC, Thomas F, Guégan JF. Evidence for phylogenetic inheritance in pathogenicity of *Mycobacterium*. *Antonie Van Leeuwenhoek* 2003; **83:** 265–74.

65 Delafont V, Bouchon D, Héchard Y, Moulin L. Environmental factors shaping cultured free-living amoebae and their associated bacterial community within drinking water network. *Water Res* 2016; **100:** 382–92.

66 Thomas V, McDonnell G. Relationships between mycobacteria and amoebae: ecological and epidemiological concerns. *Lett Appl Microbiol* 2007; **45:** 349–57.

67 Jang J, Becq J, Gicquel B, Deschavanne P, Neyrolles O. Horizontally acquired genomic islands in the tubercle bacilli. *Trends Microbiol* 2008; **16:** 303–08.

68 Dubois V, Pawlik A, Bories A, et al. *Mycobacterium abscessus* virulence traits unraveled by transcriptomic profiling in amoeba and macrophages. *PLoS Pathog* 2019; **15:** e1008069.

69 Lagune M, Petit C, Sotomayor FV, et al. Conserved and specialized functions of Type VII secretion systems in non-tuberculous mycobacteria. *Microbiology (Reading)* 2021; **167**.

70 Danelishvili L, Wu M, Stang B, et al. Identification of *Mycobacterium avium* pathogenicity island important for macrophage and amoeba infection. *Proc Natl Acad Sci U S A* 2007; **104:** 11038–43.

71 Cirillo JD, Falkow S, Tompkins LS, Bermudez LE. Interaction of *Mycobacterium avium* with environmental amoebae enhances virulence. *Infect Immun* 1997; **65:** 3759–67.

72 Hasan NA, Norton GJ, Virdi R, et al. Measurable genomic changes in *Mycobacterium avium* subsp. *hominissuis* after long-term adaptation in *Acanthamoeba lenticulata* and reduced persistence in macrophages. *J Bacteriol* 2021; **203:** e00257-20.

73 Mura M, Bull TJ, Evans H, et al. Replication and long-term persistence of bovine and human strains of *Mycobacterium avium* subsp. *paratuberculosis* within *Acanthamoeba polyphaga*. *Appl Environ Microbiol* 2006; **72:** 854–59.

74 Guerra CA, Delgado-Baquerizo M, Duarte E, et al. Global projections of the soil microbiome in the Anthropocene. *Glob Ecol Biogeogr* 2021; **30:** 987–99.