Urban parrots in Europe

Adaptations of parrot gut microbiota to city environments

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Stadsnära papegojor i Europa – Anpassningar av papegojors tarmflora i urbana miljöer

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Abstract

Invasive alien species are a problem on a global scale, as they cause damage of either environmental or economic value, or put human health at risk. The rose-ringed parakeet and the monk parakeet are two examples of species which have been classified as invasive in established populations outside of their native ranges, including continental Europe and North America. They are known to cause agricultural damage and noise pollution in both native areas and areas in which they are aliens. As they have established populations around the world, they have had to adapt to novel conditions. The purpose of this study was to investigate the gut microbiota of rose-ringed parakeets and monk parakeets in multiple European cities by analyzing fecal samples, compare and evaluate adaptations on a north-south gradient, and to assess zoonotic potential of urban parrots. This was done using fecal samples with caged monk parakeets from Sweden and soil samples as control groups.

The results were insufficient to draw conclusions regarding gut microbial adaptations on a north-south gradient. Multiple bacterial species with zoonotic potential were found in the study, indicating both species as potential disease vectors in urban environments. Further studies would need to be conducted to draw more conclusions regarding the parakeets' roles in disease transmission.

Keywords: monk parakeet, ring-rosed parakeet, gut microbiota, zoonoses, urbanization, invasive species

Sammanfattning

Invasiva arter är problematiska på en global nivå, eftersom de orsakar skada av miljörelaterat eller ekonomiskt värde. De kan också utgöra en risk för den mänskliga hälsan. Halsbandsparakiter och munkparakiter är två arter klassade som invasiva i etablerade populationer utanför deras naturliga utbredning. De etablerade populationerna finns bland annat i Europa och Nordamerika. Arterna är kända, både inom sina naturliga och introducerade utbredningar, för att orsaka skada i jordbruket, samt för att ge upphov till ljudföroreningar. När arterna spridits runt jorden har de behövt anpassa sig efter nya förhållanden och miljöer. Syftet med denna studie var att undersöka anpassningar i tarmfloran hos parakiter i europeiska städer på en nord-sydlig gradient, samt att utvärdera den zoonotiska potentialen hos urbana papegojor. Detta gjordes genom avföringsprover med svenska burhållna munkparakiter och jordprover som kontrollgrupp.

Baserat på resultaten från studien gick det inte att dra några slutsatser om anpassningar av tarmfloran på en nord-sydlig gradient. Flera bakteriella arter med zoonotisk potential upptäcktes i studien, vilket tyder på att båda arterna har potential att fungera som vektorer för sjukdomar i urbana miljöer. Vidare studier av parakiternas roller som sjukdomsvektorer behöver genomföras för att dra mer slutsatser om parakiternas roll i sjukdomsspridning.

Nyckelord: munkparakit, halsbandsparakit, tarmflora, zoonoser, urbanisering, invasiva arter

Table of contents

List of tables

List of figures

Abbreviations

Abbreviation	Description
SLU	Swedish University of Agricultural Sciences
DAISIE	Delivering Alien Invasive Species Inventories for Europe
ICUN	International Union for Conservation of Nature
PCR	Polymerase Chain Reaction
dNTPs	Deoxynucleotide Triphosphates
HF	High Fidelity
BSA	Bovine Serum Albumin

1. Introduction

1.1 Background

As urban environments spread, there is less room for wild plants and animals in their natural surroundings. When access to natural environments decreases, animals often resort to more synanthropic lifestyles in or around urban areas (Strubbe & Matthysen 2009). Some of these species are native to the area, while others are introduced either by human transportation or relocate due to environmental changes (Taylor & Irwin 2004; Da Silva *et al.* 2010; Elith *et al.* 2010; Robinson *et al.* 2020).

The rose-ringed parakeet (*Psittacula krameri*) from central Africa and southern Asia, and the monk parakeet (*Myiopsitta monachus*) from South America, have been imported to Europe as pets (Juniper & Parr 1998; Cameron 2012; Ribeiro *et al.* 2021). These parrot species have later escaped or been released across the European continent and managed to establish vital populations in, mainly, large cities in western Europe (Strubbe & Matthysen 2009).

When alien species establish in a new area, they need to adapt to the novel conditions and may disturb the order of local ecosystems. Along with the innate risk of outcompeting local species, alien species also have the potential to introduce new pathogens or to be good vectors for pre-existing diseases (Chinchio *et al.* 2020; Blanco-González *et al.* 2024). Surveying these adaptations is interesting to monitor potential routes of disease transmission, and it is also relevant for developing appropriate management strategies of invasive species.

1.2 Purpose

The purpose of this study was to examine the gut microbiota of urbanized parrots across six major European cities and caged birds in Sweden, assess differences in bacterial composition on a north/south gradient, and between wild parakeets and caged monk parakeets. The goal was to observe any signs of urban adaptations and assess zoonotic potential.

2. Literature Review

2.1 Alien species

All around the world, living organisms relocate across borders into areas previously uninhabited by their species. These relocations may be caused by human translocation, environmental changes or range expansions (Elith *et al.* 2010). Despite national and international legislation and agreements to prevent the establishment of alien species, anthropogenic introductions to new areas still happens globally at an increasing rate (Seebens *et al.* 2017; Mazza & Tricarico 2018; Chinchio *et al.* 2020). Birds are known to be among the most successful alien invaders (Blackburn *et al.* 2009) and according to the DAISIE Inventory of Alien Invasive Species in Europe, a total of 1 411 alien vertebrate species has been identified in Europe, out of which approximately one quarter are avian (326/1 411) (Roy *et al.* 2019).

Climate-matching and human-activity are two major hypotheses on establishment success of alien species (Williamson 1996; Taylor & Irwin 2004; Leprieur *et al.* 2008). The climate-matching hypothesis states that, for a new species to be successful, the new climate needs to be sufficiently comparable to their native climate (Williamson 1996). There are many different aspects of climate change that may facilitate alien species establishment in new areas (Robinson *et al.* 2020), increase the risk of species extinctions and endanger whole ecosystems (Calvin *et al.* 2023). Temperature is an important factor, placing limits on living organisms' ability to thrive in an environment. Due to the increasing temperatures caused by global warming, many species of animals and plants have been able to expand their native ranges into areas where lower temperatures would previously have been making it impossible for them to live (Walther *et al.* 2009).

The human-activity hypothesis is concerned with the impact of different aspects of human activity on the success and distribution of alien species, such as human population density and economic factors that fuel the movement and introductions of exotic and alien species into new areas (Taylor & Irwin 2004). One aspect strengthening the human-activity hypothesis is the increased likelihood of introduction supplementation through additional releases associated with higher population density, known as propagule pressure (Da Silva *et al.* 2010) which decreases the risk of an introduction failing due merely to factors of chance (Blackburn *et al.* 2009). According to Strubbe & Matthysen (2009), the establishment success of both rose-ringed parakeets (*Psittacula krameri*) and monk parakeets (*Myiopsitta monachus*) was positively associated with human population density, and negatively associated with number of frost days, meaning both parrot breeds are more likely to succeed in human-dense, warm areas.

Since the end of the 1900's, rose-ringed parakeets and monk parakeets have established healthy breeding populations in multiple areas in temperate parts of Europe, such as Italy, Spain and the Netherlands (Strubbe & Matthysen 2009; Pârâu *et al.* 2016). The rose-ringed parakeets are native to central Africa and south Asia, while Monk parakeets are native to southeastern South America (Forshaw 2010; Cameron 2012). Both species were brought to Europe to be kept as cage birds. Through both accidental escapes and deliberate releases, both species have been able to establish breeding populations, mainly in parks in larger cities. (Strubbe & Matthysen 2009; Cameron 2012; Pârâu *et al.* 2016).

2.1.1 Invasive alien species

When an alien species is introduced into a new environment and causes damage of either environmental or economic value, or puts human health at risk, it is termed an invasive species (Mazza & Tricarico 2018). Key factors in invasion success include traits of the introduced species, the site, and the propagule pressure. When alien species lack natural competition and predators, which is often the case, likelihood of invasion success increases (Rivera-Estay *et al.* 2024).

Alien species finding their niche in an ecosystem will cause disruptions to the carefully balanced interactions making the ecosystem stable and functional in the first place. The common myna (*Acridotheres tristis*) is, much like the rose-ringed parakeet, a cavity nesting bird native to southeast Asia which through both deliberate and accidental releases has been introduced to multiple areas, including Australia, New Zealand, South Africa and the United States, as well as multiple smaller islands and island groups (Stuart *et al.* 2024). In the year 2000, the common myna was selected by the ICUN as one of three avian species to be included in their list of the "100 Worst Invasive Alien Species" due to their negative impact on populations and environments (Simberloff & Rejmanek 2019). The species exhibits aggressive behavior and are very competitive regarding nesting sites, which may be a scarce resource for cavity nesters (Rogers *et al.* 2020). This may in turn negatively impact native populations as failure to find a suitable nesting site will result in a breeding pair not being able to reproduce (Charter *et al.* 2016).

The economic effects of invasiveness may be in the form of agricultural damage. Both monk- and rose-ringed parakeets are agricultural pests in their native ranges and cause damage to crops while feeding and have been observed damaging crops in their new environments as well (Clergeau & Vergnes 2011; Cameron 2012; Senar *et al.* 2016). Another example of economic damage caused by alien species is the Canada goose (*Branta canadensis*) which originates from North America but has been released in Europe for hunting purposes since the 1600s. The species has since expanded and become abundant across the continent and is known to exhibit many invasive traits. For example, Canada geese cause significant damage to crops such as wheat and corn, and damage to golf course greens, which can have considerable economic consequences (Fouque & Schricke 2011).

Human health can be put at risk both directly and indirectly through pathogen transmission to and from invasive alien species. Human health will be affected directly by new pathogens being introduced in the area by alien species which have not previously been present. Indirectly, alien species can act as reservoirs for pathogens, perhaps unable to transmit a disease directly to humans but still able to act as vectors of the disease and amplifying pathogen transmission in other populations that are then able to infect humans or affect us indirectly in other ways (Chinchio *et al.* 2020).

2.1.2 One Health

The One Health concept recognizes the health of humans, animals, and the environment as interlinked and interdependent. If one aspect of the system is disturbed, it weakens the rest and increases the risk of new diseases emerging or spreading. Human activity has a large negative impact on many aspects of the One Health concept. Therefore, it is important to mind all aspects of an ecosystem to, for example, reduce risk of sickness in humans. By integrating the different fields, One Health aims to optimize the health of all three (WHO 2023).

In relation to invasive species, the One Health concept is highly relevant as new species affect many aspects of the system they are introduced to. Since a new species carries traits and, potentially, pathogens into the new environment that may be unique and foreign, they also carry great potential to upset the system (Chinchio *et al.* 2020).

2.2 Feral parrots

In their native ranges, both rose-ringed parakeets and monk parakeets are considered agricultural pests and cause noise pollution (Clergeau & Vergnes 2011; Cameron 2012; Fraticelli 2014). In spite of the negative opinion of the birds in their native ranges, the public generally has a positive attitude toward them in the areas where they are alien (mainly urban areas), largely due to their vivid colors and their apparent harmlessness (Ribeiro *et al.* 2021).

Rose-ringed parakeets and monk parakeets are considered invasive in many areas in Europe as well as many other places around the world. In urban Seville, rose-ringed parakeets are displacing the greater noctule (*Nyctalus lasiopterus*), a species of bat with similar nesting preferences to the parakeet, by occupying suitable nests and exhibiting aggressive behavior towards them (Hernández-Brito *et al.* 2018). Monk parakeets have caused a considerable reduction in corn, plum and pear harvests in the Baix Llobregat agricultural area to the west of Barcelona, Spain (Senar *et al.* 2016). Both species are highly social and gather in large, loud flocks, especially outside of their breeding seasons, becoming a nuisance to people in the area (Juniper & Parr 1998; Forshaw 2010).

In July 2007, the European Union passed a regulation banning further imports of parakeets in hopes that this would stunt the populations' growth by reducing propagule pressure (Commission Regulation (EC) No 318/2007). The ban is believed to have had a part in increasing propagule pressure and allowed parakeet invasions to be successful. The populations had nevertheless already been sufficiently established in many areas to be able to manage the reduced number of new introductions, and the populations are still vital.

2.2.1 Rose-ringed parakeets, *Psittacula krameri*

With native ranges in central Africa and southern Asia, the rose-ringed parakeet is a relatively new addition to the European fauna, with its first reports in the late 1960's (Ribeiro *et al.* 2021). Most feral populations in Europe are of the subspecies *P. k. manillensis*, which originates from peninsular India and Sri Lanka. Other subspecies include *P. k. krameri* and *P. k. parviostris*¸ with natural ranges in western and eastern parts of Sub-Saharan Africa, respectively, as well as *P. k. borealis* from northern India, Pakistan, Nepal, Bangladesh, and Myanmar (Juniper & Parr 1998). In the wild, the species is a social and relatively sedentary bird. They tend to prefer to breed, feed and roost in large social gatherings (Juniper & Parr 1998; Forshaw 2010).

Psittacula krameri are weak excavators, commonly living in pre-existing tree holes that they can enlarge if necessary (Orchan *et al.* 2013). They breed and feed at higher elevation than many other species, which is a suggested explanation for reduced competition, as well as for fewer interspecies interactions compared to others (Fraticelli 2014; Borray-Escalante *et al.* 2020). Their diet consists of cereals, weed- and tree seeds, fruits, flowers, nectar, nuts, and leaves and is often supplemented through bird feeders in urban environments (Juniper & Parr 1998; Borray-Escalante *et al.* 2020).

2.2.2 Monk parakeets, *Myiopsitta monachus*

Monk parakeets are native to South America (Juniper & Parr 1998; Forshaw 2010) but have also been able to establish populations in Europe, the United States, the Caribbean and Japan (Da Silva *et al.* 2010). The feral subspecies most common in North America is *M. m. monachus,* native to Uruguay and Argentina. This supports the theory of the exotic pet trade as the source of feral parakeets, at least as it pertains to the North American population since this is the most common subspecies to be sold as cage birds in the region (Juniper & Parr 1998; Cameron 2012). Other subspecies of *M. monachus* include *M. m. cotorra* and *M. m. calita,* with *calita* being a slightly smaller race with ranges in Argentina, Brazil, Bolivia, and Paraguay, as well as *M. m. luchsi* living in Andean valleys in Bolivia (Juniper & Parr 1998).

Monk parakeets are nest builders and tend to favor building stick nests on human infrastructure like electrical posts, which provide solid platforms as well as heat. This is sometimes problematic as these nests are known to have caused fires and power outages (Cameron 2012). Monk parakeets are known to share both feeding and nesting sites with feral pigeons, potentially increasing risk of disease transmission (Mori *et al.* 2019). The diet of the monk parakeet in its natural ranges is quite varied, consisting of both wild and cultivated seeds, fruits and vegetables, as well as sometimes even insects and insect larvae (Juniper & Parr 1998). A study carried out in a park in Barcelona (Borray-Escalante *et al.* 2020) suggests that the diet of the feral monk parakeet consists of leaves, seeds, herbaceous plants as well as rice and bread. Rice and bread are directly anthropogenic food sources, showing that the monk parakeet has adopted a synanthropic lifestyle after becoming feralized.

2.3 Physiological role of gut microbiota

The microbiota of the gut aids in digestion, is essential for immune function and can even affect behavior. In blow flies, the bacteria *Proteus mirabilis* has an enormous impact on the flies behavior as it attracts flies to its odor while also being utilized to aid in swarming behavior (Ma *et al.* 2012). Gut microbiota composition is affected by a host of different aspects including, but not limited to, habitat, diet, and season (Dietz *et al.* 2022; Yan *et al.* 2024).

Different parts of the digestive tract contain bacteria with different traits to enable different metabolic processes to occur. Most bird species' intestinal microbes consist mainly of saccharolytic bacteria, meaning bacteria only capable of digesting simple carbohydrates (Kohl 2012). While the birds would be able to digest these substrates without the help of gut bacteria, there is evidence that the total energy absorbed by the bird is greater with the help from microbial fermentation than without it (Kohl 2012).

In contrast to mammals, who develop B lymphocytes in their bone marrow, members of the avian class produce them in a diverticulum of the cloaca called the bursa of Fabricius (Kohl 2012; König 2016). The bursa of Fabricius is a lymphoid organ which atrophies within 6 months after birth, prior to which it produces immunocompetent B lymphocytes by interacting with antigens from microbes in the gut (Glick *et al.* 1956; Glick 1983). A 2022 study found that the bacterial composition of the gut microbiota of mice was significantly correlated with their diet selection behavior (Trevelline & Kohl 2022). The microbial composition also plays a part in social communication through chemical signaling (Archie & Tung 2015).

2.4 Zoonotic bacterial diseases

A zoonotic disease is defined as a disease, microbial or parasitic in nature, that is transmitted between animals and humans (Vourc'h *et al.* 2022). When a species is introduced to a new area, it may carry new pathogens with it. If these pathogens are new to the population, it may spill over to domestic animals and even humans depending on the traits of the pathogen.

As alien species may also be susceptible to local diseases while being immunologically naïve to them, their impact on endemic disease transmission is often greater than that of native wildlife (Henriksen *et al.* 2018; Chinchio *et al.* 2020; Roy *et al.* 2023). For many reasons, wild birds, especially in the urban landscape, are important to the propagation, maintenance and transmission of zoonotic diseases (Hamer *et al.* 2012). Both wild and domesticated birds can be carriers of well-known pathogenic bacteria like *Salmonella* spp. and *Campylobacter* spp. Parrots specifically have been known to be vectors of a number of zoonotic bacterial diseases, including enteropathogenic *E. coli* (EPEC) and psittacosis (Cameron 2012; López *et al.* 2023). The spread of all diseases mentioned is possible via the fecal-oral route, as well as via contact with infected individuals (Cameron 2012; Samanta & Bandyopadhyay 2017; Bhandari *et al.* 2025; Livsmedelsverket 2025b).

2.4.1 *Salmonella* spp.

Salmonella spp. is transmitted fecal-orally and infect enterocytes in the intestine (Samanta & Bandyopadhyay 2017). The resulting disease, Salmonellosis, may be both acute and chronic. Symptoms in birds range from mortality without prior cardinal signs to gastrointestinal symptoms. Birds with acute infections show depression, anorexia, dyspnea, and diarrhea, before death occurs. Chronic form causes gastrointestinal (anorexia, diarrhea, crop stasis), respiratory (dyspnea) and ophthalmic (conjunctivitis, panophthalmitis) symptoms as well as general, non-specific symptoms like lethargy, ruffled feathers and drop in egg production (Samanta & Bandyopadhyay 2017). Symptoms in humans include diarrhea, abdominal cramps, fever, and vomiting. In some cases, people may develop arthritis as a complication of a salmonella infection (Livsmedelsverket 2025b).

2.4.2 *Campylobacter* spp.

Campylobacter spp. is a family of zoonotic bacteria that do not cause any symptoms in psittacines (Samanta & Bandyopadhyay 2017). Parrots do however have the potential to act as an asymptomatic reservoir and are then able to spread the disease to other hosts, including humans, dogs and cats (Samanta & Bandyopadhyay 2017; VetBact 2025). Human symptoms include bloody diarrhea, vomiting, fever, nausea, headaches, and intense abdominal pains. Infections may also cause arthritis, nervous symptoms and chronic gastrointestinal issues (Livsmedelsverket 2025a).

2.4.3 *Escherichia* spp.

Escherichia spp. is a bacterial family which includes both pathogenic and non-pathogenic species, the most common being *E. coli*. It is the most common bacterial species in the human digestive tract, and most strains are non-pathogenic. However, some strains are pathogenic and are known to cause a number of different clinical presentations, including food poisoning and urinary tract infections (VetBact 2023a). The pathogenic species of *E. coli* are zoonotic, and some strains of Shiga toxin-producing *E. coli* may cause Hemolytic Uremic Syndrome (HUS), a serious disease with painful diarrhea and abdominal cramping, as well as vomiting, renal failure and, in severe cases, death (Michael *et al.* 2022; Bhandari *et al.* 2025). In a 2023 study by López *et al.*, it was shown that both feral rose-ringed parakeets and monk parakeets were carriers of enteropathogenic *E. coli* (EPEC), which is zoonotic and mainly causes food poisoning symptoms in young children in low-income countries (López *et al.* 2023; VetBact 2023a).

2.4.4 Psittacosis

Psittacosis is a disease in humans caused by the bacterium *Chlamydophila psittaci*, which also causes avian chlamydiosis in birds. The disease causes nonspecific clinical signs in birds, including anorexia, decreased egg production, nasal and ocular discharge, fever, and diarrhea (Van Wettere 2025). Subclinical infections occur and are common in parrots (Van Wettere 2025).

Humans experience flu-like symptoms, including, but not limited to, fever, headaches and weakness and normally become infected through inhalation of bacteria present in droppings or other secretions coming from infected birds. The disease is treatable with antibiotics but has been known to cause fatalities (Cameron 2012). The bacterium is also known to cause ovine chlamydiosis, as well as enzootic abortion in ewes, and has hence been renamed *Chlamydia abortus* (VetBact 2023b).

3. Materials and methods

3.1 Literature search

Literature search was conducted using multiple different search engines, including Google Scholar, Web of Science, and Primo and included search phrases such as “"climate change*" AND "invasive species"”, “feralization AND (impact* OR effect*) AND (ecolog* or biolog*)”, and “("myiopsitta monachus" OR "monk parakeet" OR "psittacula krameri") AND feral* AND europe*”. Many articles referenced in this essay were found in reference lists in articles found using these search engines and phrases.

3.2 Fecal sample collection

Sample collection of feral parrots was conducted between March and August of 2025 in six locations across Europe, as well as by collecting samples from caged birds living in Sweden. The cities included in the study are, in order of date first visited, Barcelona, Spain (24/3-25 and 6/6-25); Padua, Italy (1/5-25); Madrid, Spain (6/5-25); Valencia, Spain (15-16/6-25); Amsterdam, Netherlands (13-15/6-2025); Seville, Spain (25-27/8-2025). The caged birds were kept privately in Malmö, southern Sweden (Table 1; Figure 1).

Local ranges were obtained through international birdwatching databases such as eBird (ebird.org) and observation.org and filtering for recent reports of parakeet sightings in parks, as well as through anecdotal information and personal observations. By going to parks in cities with a known population of rose-ringed parakeets and/or monk parakeets and locating nests or congregation points in the area, sample collection was conducted on the ground underneath these points by three different persons. When the sampler saw a bird defecating, the fresh droppings were collected. If no bird was seen defecating, feces appearing fresh and of similar size and shape as known parakeet samples were collected beneath gatherings of birds from the correct species. Two soil samples collected in association to bird droppings were included in the study as environmental control samples from Padua (Italy) and Barcelona (Spain), respectively.

3.2.1 Sample storage

The samples were stored in 50 ml test tubes containing Silica Gel pouches in room temperature until refrigeration or freezing was possible. In some cases, samples were stored in room temperature for up to three days before freezing was possible and some samples were stored in the freezer at -20 °C for up to six months. The three samples from the Swedish caged birds were stored in a refrigerator for 10 days before each sample was divided in two and either being frozen and dried with a silica gel pouch or being freshly frozen.

Table 1: Sample locations and dates in order of first sample date

Location	***Coordinates***	***Number of samples***	***Dates (number of samples)***	***Comments***
Barcelona, Spain	41°23'57.9"N 2°12'24.4"E & 41°23'17.1"N 2°11'10.2"E	14	24/3-2025 (3) & 6/6-2025 (11)	13 monk parakeets Includes 1 soil sample from Parque de la Ciutadella 24/3
Padua, Italy	45°24′28.69″N 11°53′9.1″E	10	1/5-2025	9 samples of unsure origin, likely rose-ringed parakeets Includes 1 soil sample
Madrid, Spain	40°23′0″N, 3°43′0″W	4	6/5-2025	4 monk parakeets
Valencia, Spain	39°28'49.8"N 0°22'04.4"W	15	15-16/5-2025	15 monk parakeets
Amsterdam, Netherlands	52°21'36.3"N 4°55'14.3"E & 52°23'09.8"N 4°52'40.4"E	10	13-15/6-2025	10 rose-ringed parakeets
Seville, Spain	37°22'27.5"N 5°59'15.8"W	13	25-27/8-2025	13 rose-ringed parakeets
Malmö, Sweden	55°35′0″N, 13°0′0″E	6	5/8-2025	3 caged monk parakeets, 1 dry sample and 1 fresh sample from each

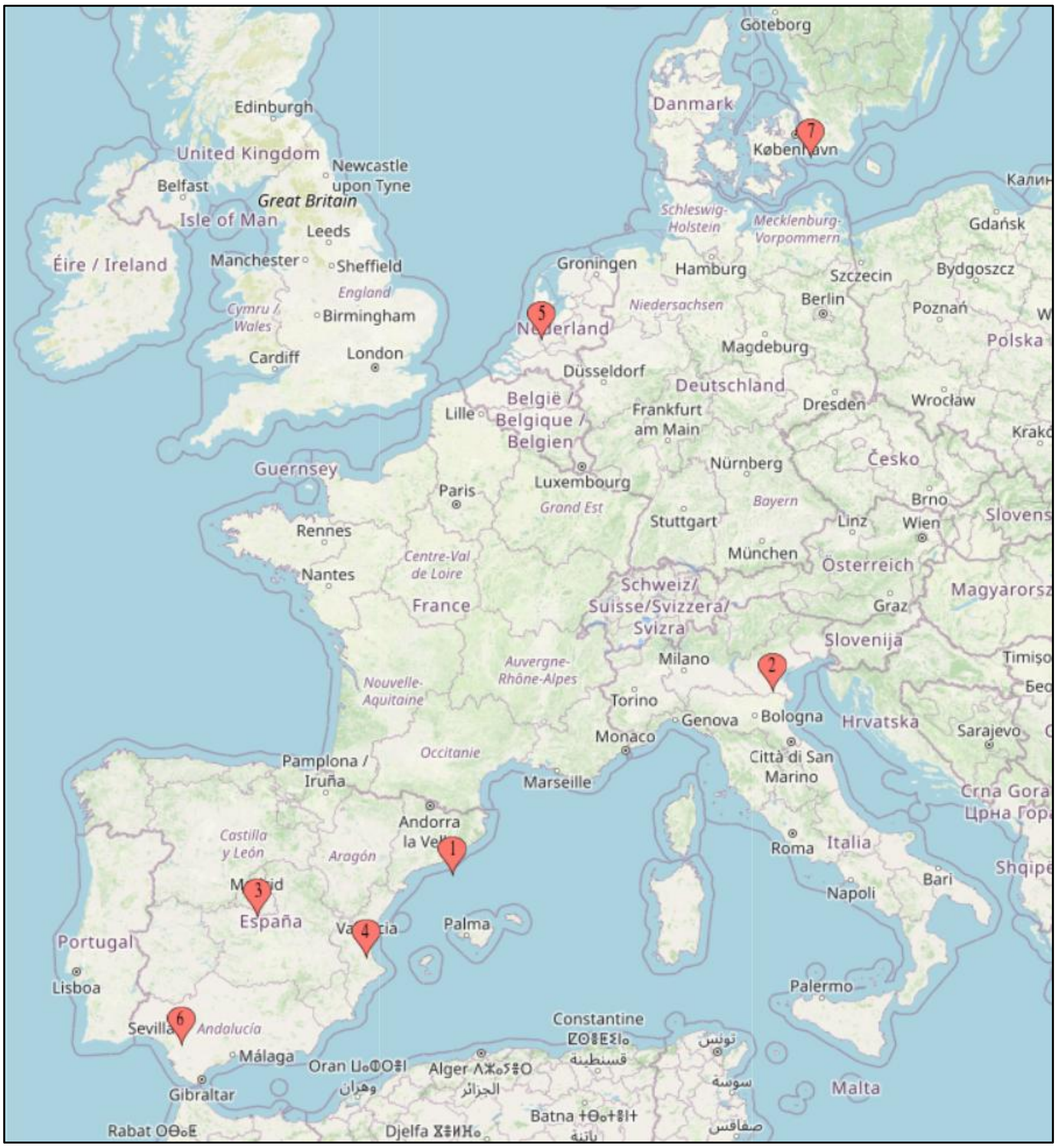

Figure 1: Map of sample locations, numbered in order of first sample date. 1: Barcelona, Spain. 2: Padua, Italy. 3: Madrid, Spain. 4: Valencia, Spain. 5: Amsterdam, Netherlands. 6: Seville, Spain. 7: Malmö, Sweden. Map created with mapcustomizer.com.

3.3 DNA purification

DNA purification from both fecal and soil samples was done using a Zymo Research Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit at SLU, Uppsala.

Frozen and dried samples up to approximately 30 mg were placed into a BashingBead™ Lysis Tube and 750 µl BashingBead™ Buffer was added. The samples were homogenized at 5000 RPM for 40 seconds, followed by a 45 second pause, repeated for four cycles. The lysate was then centrifuged at 11000 rcf for 1 minute. 400 µl supernatant was transferred onto a Zymo-Spin™ III-F Filter, placed in a DNA LowBind tube, and centrifuged at 8000 rcf for 1 minute.

1200 µl Genomic Lysis Buffer was added to the filtrate in the LowBind tube and 800 µl of the mixture was loaded onto a Zymo-Spin™ IICR Column and spun at 11000 rcf for 1 minute. Flow-through was discarded and the process was repeated with the remaining mixture. 200 µl DNA Pre-Wash Buffer was added into each Zymo-Spin™ IICR Column and spun at 11000 rcf for 1 minute, followed by adding 500 µl g-DNA Wash Buffer into each column and spinning at 11000 rcf for 1 minute. The DNA was eluded by transferring the columns onto clean DNA LowBind tubes and adding 45 µl DNA Elution Buffer directly onto the matrix, then centrifuging at 12 000 rcf for 30 sec. A Zymo-Spin™ III-HRC Filter was prepared by adding 600 µl Prep Solution and spinning at 8000 rcf for 3 minutes. The filter was placed in a clean DNA LowBind tube, and the eluded DNA was added to the filter and centrifuged at 15 000 rcf for 3 minutes.

3.4 PCR

3.4.1 Primer preparation

Primers were synthetized by Eurofins and purified using HPLC. The primers were diluted with TE buffer to a stock solution of 100 µM. The stock solutions were diluted 1:10 with water to create working solutions (e.g., 1 µl stock and 9 µl water). Primers for metabarcoding were added in an array for every individual sample to receive a unique barcode combination to be used as identification after sequencing. This was achieved by combining 16 different i5 primers with 9 i7 primers in a cross-pattern design (Appendix 1).

3.4.2 Determining number of PCR cycles

Three PCR were performed to determine optimal number of cycles for the first metabarcoding PCR. Four samples (B3, D2, F6 and E4) were included to investigate sample variation to see how many cycles to run for 515F and 806R primers: 0.2 µl dNTPs, 2 µl Buffer HF, 0.3 µl BSA, 0.125 µl Primer 515F, 0.125 µl Primer 806R, 0.1 µl Phusion Taq, 6.15 µl ddH_2O and 1 µl DNA in each well; 1 cycle of 30 seconds at 98 °C / 21, 24 and 28 cycles of 10 seconds at 98 °C, 20 seconds at 56 °C, 15 seconds at 72°C / 1 cycle of 8 minutes at 72 °C, ∞ 4 °C.

3.4.3 Bacterial 16S metabarcoding PCRs

Duplicate PCR reactions were performed to reduce the risk of individual DNA sequences taking over and becoming disproportionally replicated.

First PCR (amplification of 16S): 0.4 µl dNTPs, 4 µl Buffer HF, 0.6 µl BSA, 0.25 µl Primer 515F, 0.25 µl Primer 806R, 0.2 µl Phusion Taq 12.3 µl ddH_2O and 2 µl DNA in each well; 1 cycle of 30 seconds at 98 °C / 28 cycles of 10 seconds at 98 °C, 20 seconds at 56 °C, 15 seconds at 72 °C / 1 cycle of 8 minutes at 72 °C, ∞ 4 °C.

Second PCR (Addition of Illumina sequencing primers and barcodes to the PCR products from the first PCR): 0.4 µl dNTPs, 4 µl buffer GC, 12.8 µl Phusion Taq, 12.8 µl ddH_2O, 0.80 µl primer i5, 0.80 µl primer i7, and 1 µl PCR product from the first PCR was used as the template; 1 cycle of 30 seconds at 98 °C / 14 cycles of 10 seconds at 98 °C, 20 seconds at 56°C, 15 seconds at 72 °C / 1 cycle of 8 minutes at 72°C, ∞ 4 °C.

3.4.4 Purification, dilution, and pooling

After PCR was performed, samples were pooled by transferring 10 µl PCR product from eight samples on the PCR plate into DNA LowBind tubes, providing a volume of 80 µl. To avoid large volumetric differences, one pool from the 16S metabarcoding PCR contained product from nine samples, for a total volume of 90 µl.

The final pooled samples were purified using AMPure XP beads. 80 µl of freshly vortexed, room temperature AMPure XP bead solution was added into Eppendorf tubes containing 80 µl pooled PCR product, providing a volume ratio to the PCR product of 1:1. In the pool containing PCR products from nine different samples, 90 µl AMPure XP bead solution was added to achieve a ratio of 1:1. The tubes were then vortexed for 10 seconds and incubated at room temperature for five minutes. When five minutes had passed, they were placed into the tube rack for 5-10 minutes, or until the supernatant was clear to the naked eye. The supernatant was aspirated and discarded without disturbing the beads and 400 µl of a freshly mixed 70% ethanol solution was dispensed into each Eppendorf tube, not directly onto the beads. The tube was left to incubate for one minute before aspirating the ethanol and repeating the wash one time. After the second wash step, as much ethanol as possible was aspirated without disturbing the beads and the tube was left open to dry for at least 15 minutes. When dry, 42 µl of Tris 10mM elution buffer was added to the beads and the mixture was vortexed at a low speed until the magnetic particles were fully resuspended. The solution was left to incubate at room temperature for five minutes and was then placed back into the magnetic rack. When the solution was clear (after approximately 3 minutes), 40 µl was transferred to a fresh Eppendorf tube.

The sample concentrations from each tube were measured using a Qubit® 2.0. By pipetting specific volumes from each sample pool, equal amounts of DNA were transferred into a new tube, and the final concentration and average length of PCR products was measured on a TapeStation.

3.5 Illumina sequencing

DNA sequencing of metabarcoded PCR products was performed by the National Genomics Infrastructure (NGI), hosted by Science for Life Laboratory (SciLifeLab), Solna, Sweden, using an Illumina NextSeq 2000 with a P1 2x300pb flowcell.

3.6 Bioinformatics

The sequences delivered by SciLifeLab were demultiplexed, with each sample sequence and read direction in separate folders. To analyze what bacterial species each sample contained, the bioinformatic pipeline dada2 version 1.20 (Callahan *et al.* 2016a) which was ran in R v4.5.2 (R Core Team 2025). The function dada2::removePrimers was used initially to remove primer sequences used in the PCR process to produce the sequencing pool (Callahan *et al.* 2016b). Secondly, the number of sequence variations for each sample was calculated and both forward and reverse sequences were merged. To reduce faulty species identifications, the frequency of error was calculated after sequencing and subsequently each sequence was analyzed to ensure no PCR product hybrids were present. Finally, species identification was performed using the function assignTaxonomy in dada2 (Callahan *et al.* 2016b). A reference database of bacterial DNA sequences, SILVA Release 138.2, was downloaded from arb-silva.de (Quast *et al.* 2013; Yilmaz *et al.* 2014).

3.7 Statistical analysis

Statistical calculations carried out in R 4.5.2 (R Core Team 2025). The relative occurrence of each bacterial species from each sample was calculated using Phyloseq v1.50.0 (McMurdie & Holmes 2013). To test for diversity in the bacterial species community for each bird, Shannon diversity index was also calculated using package Phyloseq. Shannon diversity index considers both number of species and their relative abundance. A higher value indicates greater diversity and stability in the bacterial community. A Generalized Linear Model was used to investigate differences between Shannon diversity index for each sampled population with the index values as respondent variable and location as the fixed effect variable. Data visualization was done using the package ggplot2 v4.0.1 (Wickham *et al.* 2025). These data were used in determining differences in gut-bacterial composition on a north-south gradient, in comparing gut microbial composition during different times of the year in the samples where this was possible as well as in making intraspecies comparisons of gut microbial composition.

4. Results

4.1 Samples

A total of 72 separate samples were analysed in this study. Six of theses samples originated from three caged monk parakeets where each individual contributed one fresh frozen and one dried sample. 32 samples are from wild rose-ringed parakeets, and 32 samples are from wild monk parakeets. No sample location included samples from both parakeet species. Two samples were soil samples (Appendix 1).

4.2 Monk parakeets

For wild monk parakeets (n=33), the bacterial phyla Pseudomonadota and/or Bacillota ranged between 69.8%-89.9% of the total relative abundance in samples across all locations, except from the two samples from Parque de la Ciutadella, Barcelona, where they represented 44.5%. The Parque de la Ciutadella samples did on the other hand include 14 different phyla with a relative abundance >1%, whereas the other locations with monk parakeets included only 2-6 phyla with a relative abundance >1%. Phyla with less than 5% of the total relative abundance made up 8% in Valencia, 3.2% in Madrid, 19.9% in Parque de la Ciutadella, Barcelona, 5.2% in Plaça del Prim, Barcelona, 4.4% in dried samples from Malmö and 1.5% in fresh samples from Malmö (Figure 2). No significant differences in diversity were demonstrated within the wild monk parakeet samples (Figure 3).

The relative abundance of phyla in caged Swedish monk parakeets (n=3) was similar between the fresh and dried samples, consisting predominantly of Bacillota (87.3% for the dried sample and 76.1% for the fresh sample). The fresh samples had more than twice the mean relative abundance of Pseudomonadota compared to the dry samples (22.3% and 8.3%, respectively). For both sample groups, Actinomycetota was the third most abundant, at 2.1% for the dried samples but <1% for the fresh samples. Other phyla represented <1% in both groups, giving the samples from caged monk parakeets a lower Shannon score than the wild monk parakeet samples (Figure 2; Figure 3).

4.3 Rose-ringed parakeets

All rose-ringed parakeet samples (n=33) consisted of ≥52.2% bacteria from the phylum Pseudomonadota (52.2-57.1%), regardless of sample location. Actinomycetota accounted for 10.3-16.7% of relative abundance, and Bacillota accounted for 8.1-20.8%. In the Paduan samples, 11.1% of relative abundance included Bacteroidota, whereas the phylum only stood for 2.6% and 3.7% in Amsterdam and Seville, respectively. The Sevillian samples had a relative abundance of Cyanobacteriota of 8.2%, represented by 3.9% in Amsterdam and 4.4% in Padua. Phyla with less than 5% of total relative abundance made up 10.2% in Seville, 14.9% in Padua, and 10.5% in Amsterdam (Figure 2). No significant differences in diversity were demonstrated within the species (Figure 3).

4.4 Soil samples

The soil samples were comprised of mainly Actinomycetota and Pseudomonadota. The Paduan sample consisted of 40.6% Actinomycetota and 15.9% Pseudomonadota, and the sample from Parque de la Ciutadella, Barcelona, was made up of 27.2% Pseudomonadota and 26.3% Actinomycetota. Both samples included five phyla with more than 5% relative abundance. 22.9% of the Barcelonan and 21.2% the Paduan samples consisted of phyla with less than 5% of total relative abundance (Figure 2).

4.5 Zoonotic bacteria

A total of 12 samples (17.4%), 11 from urban parrots and one from a caged parakeet, contained evidence of bacterial genomic mass belonging to species with zoonotic potential, such as *Clostridium perfringens*, a common cause of food poisoning in humans, that was detected in a total of seven samples from two different locations. *Campylobacter upsaliensis* was found in five samples, one of which was a caged monk parakeet. One Valencian monk parakeet sample included DNA from *Escherichia albertii*, a close relative of *Escherichia coli* known to cause similar symptoms to enteropathogenic or enterohemorrhagic *Escherichia coli.* In one Barcelonian sample, *Chlamydia abortus* was detected, the causative agent in psittacosis and avian chlamydiosis. No subspecies of *Salmonella* spp. was detected in any sample (Table 2).

4.6 Shannon diversity

The Shannon diversity index for the bacterial species found in the samples in this study showed no significant differences in species diversity between cities, apart from the parakeet samples from Padua and Valencia, where the Paduan samples were more diverse ($p=0.00755$) (Figure 3).

Table 2: Zoonotic bacteria detected.
Code explanation: PK/MM/US/DIRT = suspected P. krameri, M. monachus, sample of unsure origin or dirt sample, followed by location, sample year and sample number.
Example: First suspected P. krameri from Madrid, 2025 would become PKMad251

Species	Samples (n)	Locations
Clostridium perfringens	PKAms252, PKAms253, PKAms256, PKAms259, MMBar257, MMBar2510, MMBar2511 (7)	Amsterdam, NL; Plaça del Prim, Barcelona, SP
Campylobacter upsaliensis	MMVal2513, PKAms252, PKAms253, USPad2510, MMSwe255 (5)	Valencia, SP; Amsterdam, NL; Padua, IT; Malmö, SE
Escherichia albertii	MMVal2512 (1)	Valencia, SP
Chlamydia abortus	MMBar2512 (1)	Parque de la Ciutadella, SP

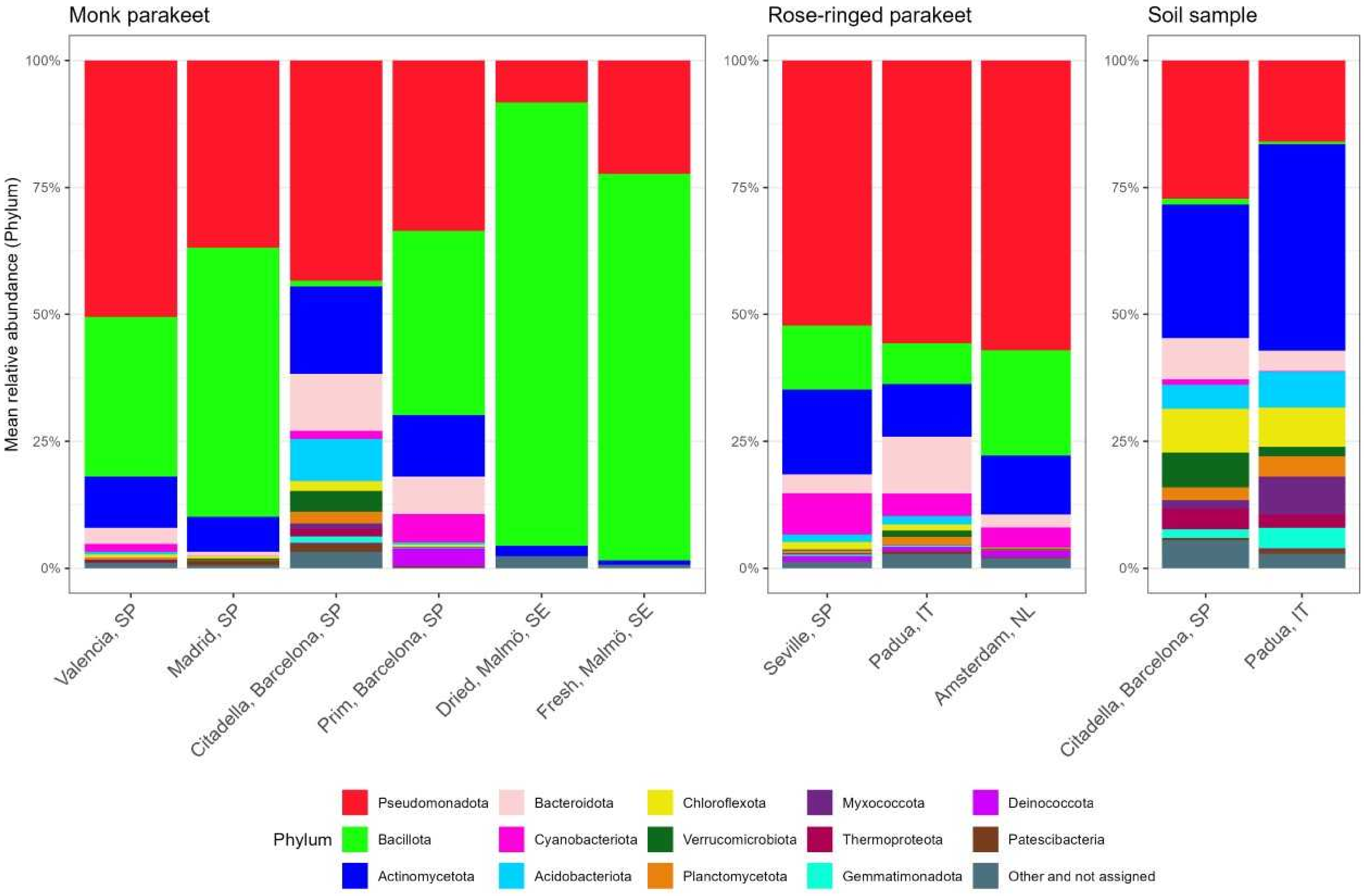

Figure 2: Mean relative abundance of bacterial phyla in fecal samples of monk parakeets, rose-ringed parakeets, and soil samples.

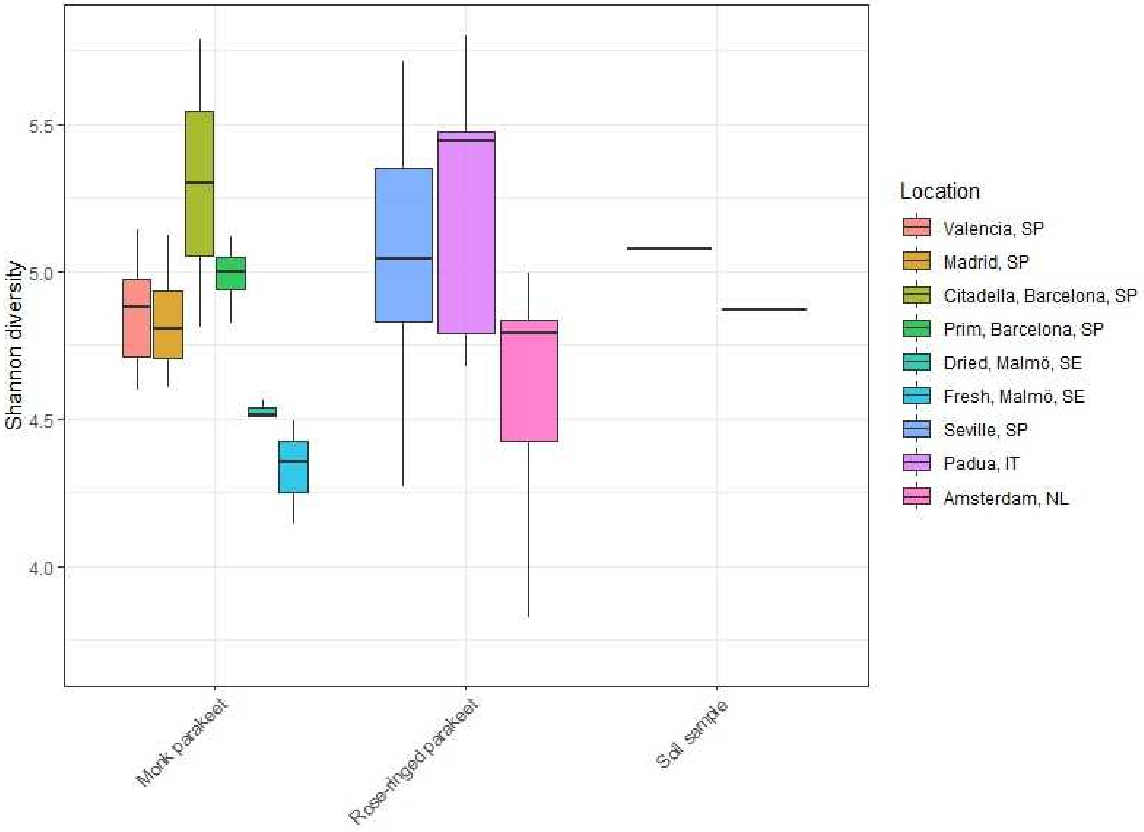

Figure 3: Box plot showing the Shannon diversity index for the two parakeet species and the soil samples. For the monk parakeets, the domestic samples from Malmö. Sweden showed the lowest diversity and the Parque de la Ciutadella the highest. The Rose-ringed parakeet in Padua had the highest median diversity of all investigated locations.

5. Discussion

5.1 Species differences

One of the most abundant phyla for both species was Pseudomonadota. The rose-ringed parakeet samples consisted of relative Pseudomonadota abundances of more than 50% across all locations. While the phylum made up a significant proportion of the total in monk parakeet samples, only the monk parakeet samples from Valencia had a relative abundance of more than half (50.5%). In wild monk parakeets, relative Pseudomonadota abundances were lowest in Plaça del Prim, Barcelona, with 33.5% of the total abundance. This may be due to a host of different factors but seeing as the phyla within either species share similar relative abundances regardless of geographical location, it is likely that the reason is related to differences in the species' preferences and behavior. This could be factors like specific dietary preferences, or likelihood of interactions with other species in the urban environment, including humans.

For monk parakeets, the relative abundance of Bacillota varied greatly among locations, from 53% in Madrid to 1.1% in Parque de la Ciutadella. Since phylum abundances included in the results of this study were relative, differences in abundance may in this case simply be attributed to differences in concentrations of bacterial species, where two samples that have the same number of total bacteria within one phylum may vary greatly in relative abundances due to differences in number of bacteria in other phyla. This means that a more diverse gut microbiota may affect the relative abundance of bacterial phyla, but as phyla generally include many bacterial species with different traits, a diverse microbiota may also present with larger relative abundances of singular phyla. It is unclear whether the large differences in relative abundance for the phylum Bacillota in monk parakeets have arisen because of seasonal differences in gut microbiota, environmental effects or something else unaccounted for in this study. Further investigations with larger sample groups from all locations and sampling occurring in closer succession are needed to dissolve these apparent differences.

The phylum Pseudomonadota is diverse and includes bacterial species with a wide variety of characteristics and natural ranges. A few examples of genera belonging to Pseudomonadota found in this study include *Sphingomonas* spp. and *Escherichia* spp. *Sphingomonas* spp. are generally not human pathogens but may be an opportunistic pathogen in immuno-compromised individuals (Chandra & Kronenberg 2015) and *Escherichia* spp. make up the most common bacteria in the human gut microbiota and includes mostly non-pathogenic species. However, the genus does include species such as *E. coli* and *E. albertii*, the latter found in this study, which are both known to include strains with potential to be zoonotic and cause severe illness in humans and animals alike. Thus, the observations in this study indicate a value in monitoring these bacteria in urban birds to increase prepared-ness for zoonotic disease outbreaks, which may originate from wild birds in urban environments.

The interspecies disparity in phylum dominance may be attributed to differences in both lifestyle and diet. While both species' diets are similar categorically, the locations where they forage for food differs, as the rose-ringed parakeet tends to prefer feeding sites at higher elevations than the monk parakeet (Cameron 2012; Fraticelli 2014; Mori *et al.* 2019). This leads to a discrepancy in microbial contacts as it is likely that the elevation difference leads to interactions with members of different animal species, as well as a likely difference in what food sources are most abundant at different elevations. The monk parakeet for example, with a preferred elevation closer to the ground than the rose-ringed parakeet, may be more likely to eat fruits and seeds which grow close to the ground or fall to the ground. In an urban environment, they likely eat human foods that have been dropped on the ground or that are placed at human heights more frequently than the rose-ringed parakeet which spends more time at higher elevations. Interspecies interactions between parakeets and other non-avian species are also likely to happen more often in the case of the urban monk parakeet compared to the rose-ringed parakeet as a wider variety of urbanized species live close to the ground (Mori *et al.* 2019; Sweet *et al.* 2022).

While there was a statistically significant difference between Paduan and Valencian diversity, this comparison is not relevant to the aim of this study as the locations included samples from different species.

5.2 North-south adaptations

No significant differences in bacterial species diversity were found in this study, with the exception that Padua had a higher Shannon diversity index than Valencia (p=0.00755) (Figure 3). This may reflect a lack of north/south gradient but may also be an effect of small sample sizes of most sample groups. The sample locations also include different parakeet species, with Paduan rose-ringed parakeets and Valencian monk parakeets. It is not clear based on these results whether this is indicative of north/south adaptations or merely species differences. Thus, it is not possible to draw conclusions regarding gut microbial adaptations on a north-south gradient based on the results of this study. Because of differences in local climate, flora, and fauna, differences in microbial composition of the gut in a north/south gradient would be likely. To be able to draw substantial conclusions about this, studies with larger sample groups would need to be conducted, sampling large groups in multiple locations in close temporal succession.

5.3 Seasonal differences

As confirmed by Dietz *et al.* (2022), gut microbiome composition may be affected by seasonal factors such as diet, temperature and day length. Given that sample collection was carried out over nearly six months, seasonal changes may have affected the results as different bacteria may have been more prominent in the gut of the parakeets during different times of the year (Figure 2; Table 1).

The samples from monk parakeets from Parque de la Ciutadella, Barcelona, were collected in late March 2025. These samples had a mean relative abundance much more similar to the corresponding soil sample than the rose-ringed parakeet samples from Padua did compared to the Paduan soil sample. An explanation for the similarity of the Barcelona samples might be that the fecal samples were contaminated with soil.

The results of this study do appear to support the theory on gut microbial composition differences in different parts of the year, although the study includes too small of a sample size to make statistically significant assumptions. While this difference may be explained by seasonal changes in gut microbiota, it could also be caused by environmental contamination, or a decreased likelihood of bacterial survival outside of the host because of factors such as temperature or humidity. Further studies would need to be performed, analyzing gut microbiota in the same area over time, in order to draw any relevant conclusions.

5.4 Wild versus caged birds

For the frozen samples from caged monk parakeets, *Bacillota* and *Pseudomonadota* made up 95.6% of total relative abundance, as compared to wild monk parakeets, where their combined relative abundance ranged from 44.5-89.9%. This may be indicative of a less diverse gut microbiota in caged monk parakeets, as also indicated in Figure 2 & Figure 3. Interestingly, many previous studies comparing wild and captive birds have found an opposing relationship, with an unaffected or more diverse gut microbiota in captive birds than in their wild counterparts (Xenoulis *et al.* 2010; Oliveira *et al.* 2020). The samples from captive monk parakeets in this test were stored in a refrigerator for 10 days before they were frozen. The days in the refrigerator might have provided a beneficial environment for one or a few bacterial species, allowing them to grow disproportionately and affect the overall relative abundance in the samples. The main reasons to suspect a decreased microbial diversity in captive birds include more uniform diet, fewer interactions with other individuals, and stress (Bajinka *et al.* 2020; Oliveira *et al.* 2020). The increase in human-animal interactions may, however, force the caged birds into interactions with the human bacterial flora, which they are much less likely to be in contact with in the wild. To further examine this relationship, studies focused on comparing wild and captive parakeets need to be performed, looking at factors such as diet, inter- and intraspecies interactions, and stress.

5.5 Zoonotic potential

Out of a total of 72 samples (69 individual samples), 12 contained bacteria with zoonotic potential. Seven samples contained *C. perfringens,* which is pathogenic and known to cause food poisoning in humans if sufficient doses are ingested, as well as gas gangrene. *C. upsaliensis* was discovered in five samples. The most common presentation for an infection with *C. upsaliensis* is enteritis, but it may also cause abortions, bacteremia and hemolytic uremic syndrome (Rowe & Madden 2014). One sample contained *E. albertii*, which is known to cause similar symptoms to enteropathogenic or enterohemorrhagic *E. coli*, and one sample contained *C. abortus*, the causative agent in psittacosis and avian chlamydiosis. Thus, it appears urban parrots do have potential to act as sources of contagion as they carry bacterial strains known to cause disease in both humans and animals. Some of these strains, such as *C. perfringens,* require large infectious doses to cause disease, which may not be achieved merely through contact with parrots and parrot feces. However, this study investigates the presence of bacterial species and does not include any quantitative analyses. This means that there may be samples included in the study which have high concentrations of pathogenic bacteria with potential to cause disease, while others may include small amounts and be highly unlikely to cause disease. To investigate this further, quantitative studies need to be made analyzing the bacterial load of zoonotic bacteria in parrot feces.

In a One Health context, the presence of bacteria with zoonotic potential means that urban parakeets may pose a health challenge, putting strain on the health of humans, animals, plants and ecosystems. The application of the One Health approach in the case of urban parakeets may be most relevant at the subnational or national levels, as the parakeets mainly can spread disease through direct or indirect contact. However, as humans travel, sickness caused by parakeets may spread further than parakeet ranges expand and the One Health approach may be applicable on a global level.

5.6 Soil samples

5.6.1 Padua

The comparison of the Paduan soil sample to the parakeet samples from the same location reveal differences in mean relative abundance of bacterial phyla. The Paduan rose-ringed parakeets' microbiota primarily consisted of bacteria from the phylum *Pseudomonadota*, whereas the largest phylum in the soil sample was *Actinomycetota*. *Pseudomonadota* is a phylum containing both environmental and animal-related bacteria. This may explain why the *Pseudomonadota* abundance is higher in parakeet samples but still present and relatively abundant in the soil sample.

Bacillota, the fourth largest group in rose-ringed parakeets at 8.1%, represented <1% of bacterial phyla in the soil sample. The reason for this is likely that the phylum contains many bacterial species common in the gastrointestinal tract of animals, and less environmental bacteria than other phyla. Many *Bacillota* can produce endospores which are resistant to the environment, making environmental *Bacillota* plausible. However, endospores do not reproduce, and other bacteria would be more likely to dominate the soil sample.

5.6.2 Parque de la Ciutadella, Barcelona

The soil samples from Parque de la Ciutadella included a diverse spread of bacterial phyla, with 22.9% of total mean relative abundance represented by phyla with <5% abundance and a total of 17 detected phyla with less than 5% abundance.

Verrucomicrobiota is a small phylum containing mainly environmental bacteria, but also includes species found in human feces. The genera identified in this study were all environmental, and their presence in parrot samples was likely caused by sample contamination. In the soil sample from Parque de la Ciutadella, Barcelona, the phylum represented 6.9% while the mean relative abundance of *Verrucomicrobiota* in rose-ringed parakeets from the same location was 4.1%. This is more than twice the abundance in the Paduan soil sample, which included 1.9% *Verrucomicrobiota*, and more than 16 times more than the average abundance for parakeets across all other locations. It is likely that the fecal samples from Parque de la Ciutadella were old and contaminated with environmental bacteria, which is further strengthened since all sample pools of wild birds contained some levels of *Verrucomicrobiota* (0.03-1.2%), while the caged birds had no detectable levels of the phylum.

5.7 Conclusions

Genomic analysis of fecal samples from the parrots included in this study shows presence of bacteria with potential to cause zoonotic disease. From the data presented in this study, no significant conclusions could be made about north-south adaptations of the gut microbiota of the monk- and rose-ringed parakeet in European city environments and caged Swedish monk parakeets. I do however show that there is considerable zoonotic potential among urban parrots in continental Europe, since 12 out of 69 individual samples included in the study contained zoonotic bacterial strains.

The study is only concerned with the qualitative analysis of bacterial genomic mass and does not take factors such as traits of disease spread or inter- and intraspecies relationships into consideration. Further studies need to be carried out to investigate the role of parakeets as disease vectors and their impact on the environments they inhabit.

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References

Archie, E.A. & Tung, J. (2015). Social behavior and the microbiome. *Current Opinion in Behavioral Sciences*, 6, 28–34. https://doi.org/10.1016/j.cobeha.2015.07.008

Bajinka, O., Tan, Y., Abdelhalim, K.A., Özdemir, G. & Qiu, X. (2020). Extrinsic factors influencing gut microbes, the immediate consequences and restoring eubiosis. *AMB Express*, 10, 130. https://doi.org/10.1186/s13568-020-01066-8

Bhandari, J., Rout, P. & Sedhai, Y.R. (2025). Hemolytic Uremic Syndrome. In: *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK556038/ [2025-10-08]

Blackburn, T.M., Lockwood, J.L. & Cassey, P. (2009). *Avian Invasions: The Ecology and Evolution of Exotic Birds*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199232543.001.0001

Blanco-González, J., López-Rull, I., Cayuela, L., Majó, N., Busquets, N., Montalvo, T. & Senar, J.C. (2024). Native and invasive bird interactions increase the spread of Newcastle disease in urban environments. *Biological Invasions*, 26 (3), 845–855. https://doi.org/10.1007/s10530-023-03213-1

Borray-Escalante, N.A., Mazzoni, D., Ortega-Segalerva, A., Arroyo, L., Morera-Pujol, V., González-Solís, J. & Senar, J.C. (2020). Diet assessments as a tool to control invasive species: comparison between Monk and Rose-ringed parakeets with stable isotopes. *Journal of Urban Ecology*, 6 (1). https://doi.org/10.1093/jue/juaa005

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016a). DADA2: High resolution sample inference from Illumina amplicon data. *Nature Methods*, 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016b). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869

Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P.W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Cheung, W.W.L., Connors, S., Denton, F., Diongue-Niang, A., Dodman, D., Garschagen, M., Geden, O., Hayward, B., Jones, C., Jotzo, F., Krug, T., Lasco, R., Lee, Y.-Y., Masson-Delmotte, V., Meinshausen, M., Mintenbeck, K., Mokssit, A., Otto, F.E.L., Pathak, M., Pirani, A., Poloczanska, E., Pörtner, H.-O., Revi, A., Roberts, D.C., Roy, J., Ruane, A.C., Skea, J., Shukla, P.R., Slade, R., Slangen, A., Sokona, Y., Sörensson, A.A., Tignor, M., Van Vuuren, D., Wei, Y.-M., Winkler, H., Zhai, P., Zommers, Z., Hourcade, J.-C., Johnson, F.X., Pachauri, S., Simpson, N.P., Singh, C., Thomas, A., Totin, E., Arias, P., Bustamante, M., Elgizouli, I., Flato, G., Howden, M., Méndez-Vallejo, C., Pereira, J.J., Pichs-Madruga, R., Rose, S.K., Saheb, Y., Sánchez Rodríguez, R., Ürge-Vorsatz, D., Xiao, C., Yassaa, N., Alegría, A., Armour, K., Bednar-Friedl, B., Blok, K., Cissé, G., Dentener, F., Eriksen, S., Fischer, E., Garner, G., Guivarch, C., Haasnoot, M., Hansen, G., Hauser, M., Hawkins, E., Hermans, T., Kopp, R., Leprince-Ringuet, N., Lewis, J., Ley, D., Ludden, C., Niamir, L., Nicholls, Z., Some, S., Szopa, S., Trewin, B., Van Der Wijst, K.-I., Winter, G., Witting, M., Birt, A., Ha, M., Romero, J., Kim, J., Haites, E.F., Jung, Y., Stavins, R., Birt, A., Ha, M., Orendain, D.J.A., Ignon, L., Park, S., Park, Y., Reisinger, A., Cammaramo, D., Fischlin, A., Fuglestvedt, J.S., Hansen, G., Ludden, C., Masson-Delmotte, V., Matthews, J.B.R., Mintenbeck, K., Pirani, A., Poloczanska, E., Leprince-Ringuet, N. & Péan, C. (2023). *IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.* Intergovernmental Panel on Climate Change (IPCC). https://doi.org/10.59327/IPCC/AR6-9789291691647

Cameron, M. (2012). *Parrots: The Animal Answer Guide*. Johns Hopkins University Press. http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=3318602 [2025-09-15]

Chandra, S. & Kronenberg, M. (2015). Activation and Function of iNKT and MAIT Cells. In: *Advances in Immunology*. Academic Press. 145–201. https://doi.org/10.1016/bs.ai.2015.03.003

Charter, M., Izhaki, I., Ben Mocha, Y. & Kark, S. (2016). Nest-site competition between invasive and native cavity nesting birds and its implication for conservation. *Journal of Environmental Management*, 181, 129–134. https://doi.org/10.1016/j.jenvman.2016.06.021

Chinchio, E., Crotta, M., Romeo, C., Drewe, J.A., Guitian, J. & Ferrari, N. (2020). Invasive alien species and disease risk: An open challenge in public and animal health. *PLOS Pathogens*, 16 (10), e1008922. https://doi.org/10.1371/journal.ppat.1008922

Clergeau, P. & Vergnes, A. (2011). Bird feeders may sustain feral Rose-ringed parakeets Psittacula krameri in temperate Europe. *Wildlife Biology*, 17 (3), 248–252. https://doi.org/10.2981/09-092

Commission Regulation (EC) No 318/2007 of 23 March 2007 laying down animal health conditions for imports of certain birds into the Community and the quarantine conditions thereof (2007). The European Union. EUR-Lex Access to European Union law. https://eur-lex.europa.eu/eli/reg/2007/318/oj [2025-10-01]

Da Silva, A.G., Eberhard, J.R., Wright, T.F., Avery, M.L. & Russello, M.A. (2010). Genetic evidence for high propagule pressure and long-distance dispersal in monk parakeet (Myiopsitta monachus) invasive populations. *Molecular Ecology*, 19 (16), 3336–3350. https://doi.org/10.1111/j.1365-294X.2010.04749.x

Dietz, M.W., Matson, K.D., Versteegh, M.A., van der Velde, M., Parmentier, H.K., Arts, Joop.A.J., Salles, J.F. & Tieleman, B.I. (2022). Gut microbiota of homing pigeons shows summer–winter variation under constant diet indicating a substantial effect of temperature. *Animal Microbiome*, 4 (1), 64. https://doi.org/10.1186/s42523-022-00216-6

Elith, J., Kearney, M. & Phillips, S. (2010). The art of modelling range-shifting species. *Methods in Ecology and Evolution*, 1 (4), 330–342. https://doi.org/10.1111/j.2041-210X.2010.00036.x

Forshaw, J.M. (2010). *Parrots of the World*. Princeton University Press. https://doi.org/10.1515/9781400836208

Fouque, C. & Schricke, V. (2011). Status and trends of the Canada Goose Branta canadensis in France. *Ornis Svecica*, 21 (2–4), 69–78. https://doi.org/10.34080/os.v21.22601

Fraticelli, F. (2014). The rose-ringed parakeet Psittacula krameri in a urban park: Demographic trend, interspecific relationships and feeding preferences (Rome, central Italy). *Avocetta*, 38 (1), 23–28

Glick, B. (1983). Chapter 6 - Bursa of Fabricius. In: Farner, D.S., King, J.R., & Parkes, K.C. (eds) *Avian Biology*. Academic Press. 443–500. https://doi.org/10.1016/B978-0-12-249407-9.50015-6

Glick, B., Chang, T.S. & Jaap, R.G. (1956). The bursa of Fabricius and antibody production. *Poultry Science*, 35 (1), 224–225. https://doi.org/10.3382/ps.0350224

Hamer, S.A., Lehrer, E. & Magle, S.B. (2012). Wild birds as sentinels for multiple zoonotic pathogens along an urban to rural gradient in Greater Chicago, Illinois. *Zoonoses and Public Health*, 59 (5), 355–364. https://doi.org/10.1111/j.1863-2378.2012.01462.x

Henriksen, R., Gering, E. & Wright, D. (2018). Feralisation—The Understudied Counterpoint to Domestication. In: Pontarotti, P. (ed.) *Origin and Evolution of Biodiversity*. Springer International Publishing. 183–195. https://doi.org/10.1007/978-3-319-95954-2_11

Hernández-Brito, D., Carrete, M., Ibáñez, C., Juste, J. & Tella, J.L. (2018). Nest-site competition and killing by invasive parakeets cause the decline of a threatened bat population. *Royal Society Open Science*, 5 (5), 172477. https://doi.org/10.1098/rsos.172477

Juniper, T. & Parr, M. (1998). *Parrots: a guide to the parrots of the world*. Pica.

Kohl, K.D. (2012). Diversity and function of the avian gut microbiota. *Journal of Comparative Physiology B*, 182 (5), 591–602. https://doi.org/10.1007/s00360-012-0645-z

König, H.E. (2016). *Avian Anatomy 2nd Edition: Textbook and Colour Atlas*. 5m Publishing Ltd. http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=4841530 [2025-10-08]

Leprieur, F., Beauchard, O., Blanchet, S., Oberdorff, T. & Brosse, S. (2008). Fish invasions in the world's river systems: When natural processes are blurred by human activities. *PLoS Biology*, 6 (12), 2940–2940. https://doi.org/10.1371/journal.pbio.0060322

Livsmedelsverket (2025a). *Campylobacter*. https://www.livsmedelsverket.se/livsmedel-och-innehall/bakterier-virus-parasiter-och-mogelsvampar1/bakterier/campylobacter/ [2025-10-13]

Livsmedelsverket (2025b). *Salmonella*. https://www.livsmedelsverket.se/livsmedel-och-innehall/bakterier-virus-parasiter-och-mogelsvampar1/bakterier/salmonella/ [2025-10-13]

López, J., Mogedas, M., Ballesteros, C., Martín-Maldonado, B., Sacristán, I., García, R., Ortiz, J.C. & Esperón, F. (2023). Infectious agents present in monk parakeet (Myiopsitta monachus) and rose-ringed parakeet (Psittacula krameri) invasive species in the parks of Madrid and Seville, Spain. *Frontiers in Veterinary Science*, 10. https://doi.org/10.3389/fvets.2023.1162402

Ma, Q., Fonseca, A., Liu, W., Fields, A.T., Pimsler, M.L., Spindola, A.F., Tarone, A.M., Crippen, T.L., Tomberlin, J.K. & Wood, T.K. (2012). Proteus mirabilis interkingdom swarming signals attract blow flies. *The ISME Journal*, 6 (7), 1356–1366. https://doi.org/10.1038/ismej.2011.210

Mazza, G. & Tricarico, E. (2018). *Invasive Species and Human Health*. CABI. (CABI Invasives series; 10)

McMurdie, P.J. & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE*, 8 (4), e61217. https://doi.org/10.1371/journal.pone.0061217

Michael, M., Bagga, A., Sartain, S.E. & Smith, R.J.H. (2022). Haemolytic uraemic syndrome. *The Lancet*, 400 (10364), 1722–1740. https://doi.org/10.1016/S0140-6736(22)01202-8

Mori, E., Sala, J.P., Fattorini, N., Menchetti, M., Montalvo, T. & Senar, J.C. (2019). Ectoparasite sharing among native and invasive birds in a metropolitan area. *Parasitology Research*, 118 (2), 399–409. https://doi.org/10.1007/s00436-018-6174-2

Oliveira, B.C.M., Murray, M., Tseng, F. & Widmer, G. (2020). The fecal microbiota of wild and captive raptors. *Animal Microbiome*, 2, 15. https://doi.org/10.1186/s42523-020-00035-7

Orchan, Y., Chiron, F., Shwartz, A. & Kark, S. (2013). The complex interaction network among multiple invasive bird species in a cavity-nesting community. *Biological Invasions*, 15 (2), 429–445. https://doi.org/10.1007/s10530-012-0298-6

Pârâu, L.G., Strubbe, D., Mori, E., Menchetti, M., Ancillotto, L., Kleunen, A.V., White, R.L., Luna, Á., Hernández-Brito, D., Louarn, M.L., Clergeau, P., Albayrak, T., Franz, D., Braun, M.P., Schroeder, J. & Wink, M. (2016). Rose-ringed parakeet Psittacula krameri populations and numbers in Europe: A complete overview. *The Open Ornithology Journal*, 9 (1), 1–13. https://doi.org/10.2174/1874453201609010001

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41 (Database issue), D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team (2025). *R: The R Project for Statistical Computing*. https://www.r-project.org/ [2025-10-27]

Ribeiro, J., Carneiro, I., Nuno, A., Porto, M., Edelaar, P., Luna, Á. & Reino, L. (2021). Investigating people's perceptions of alien parakeets in urban environments. *European Journal of Wildlife Research*, 67 (3), 45. https://doi.org/10.1007/s10344-021-01487-1

Rivera-Estay, V., Córdova-Lepe, F., Moreno-Gómez, F.N., Benitez, H. & Gutiérrez, R. (2024). Exploring the effects of competition and predation on the success of biological invasion through mathematical modeling. *Scientific Reports*, 14 (1), 4416. https://doi.org/10.1038/s41598-024-53344-1

Robinson, T.B., Martin, N., Loureiro, T.G., Phikolomzi, M. & Robertson, M.P. (2020). Double trouble: the implications of climate change for biological invasions. *NeoBiota*, 62, 463–487. https://doi.org/10.3897/neobiota.62.55729

Rogers, A.M., Griffin, A.S., van Rensburg, B.J. & Kark, S. (2020). Noisy neighbours and myna problems: Interaction webs and aggression around tree hollows in urban habitats. *Journal of Applied Ecology*, 57 (10), 1891–1901. https://doi.org/10.1111/1365-2664.13698

Rowe, M.T. & Madden, R.H. (2014). Campylobacter | Introduction. In: *Encyclopedia of Food Microbiology*. Elsevier. 351–356. https://doi.org/10.1016/B978-0-12-384730-0.00052-5

Roy, D., Alderman, D., Anastasiu, P., Arianoutsou, M., Augustin, S., Bacher, S., Başnou, C., Beisel, J.-N., Bertolino, S., Bonesi, L., Bretagnolle, F., Chapuis, J.L., Chauvel, B., Chiron, F., Clergeau, P., Cooper, J., Cunha, T., Delipetrou, P., Desprez-Loustau, M.-L., Détaint, M., Devin, S., Didžiulis, V., Essl, F., Galil, B.S., Genovesi, P., Gherardi, F., Gollasch, S., Hejda, M., Hulme, P.E., Josefsson, M., Kark, S., Kauhala, K., Kenis, M., Klotz, S., Kobelt, M., Kühn, I., Lambdon, P.W., Larsson, T.-B., Lopez-Vaamonde, C., Lorvelec, O., Marchante, H., Minchin, D., Nentwig, W., Occhipinti-Ambrogi, A., Olenin, S., Olenina, I., Ovcharenko, I., Panov, V.E., Pascal, M., Pergl, J., Perglová, I., Pino, J., Pyšek, P., Rabitsch, W., Rasplus, J.-Y., Rathod, B., Roques, A., Roy, H., Sauvard, D., Scalera, R., Shiganova, T.A., Shirley, S., Shwartz, A., Solarz, W., Vilà, M., Winter, M., Yésou, P., Zaiko, A., Adriaens, T., Desmet, P. & Reyserhove, L. (2019*). DAISIE - Inventory of alien invasive species in Europe. Version 1.7.* Research Institute for Nature and Forest (INBO). [Checklist dataset]. https://doi.org/10.15468/ybwd3x

Roy, H.E., Tricarico, E., Hassall, R., Johns, C.A., Roy, K.A., Scalera, R., Smith, K.G. & Purse, B.V. (2023). The role of invasive alien species in the emergence and spread of zoonoses. *Biological Invasions*, 25 (4), 1249–1264. https://doi.org/10.1007/s10530-022-02978-1

Samanta, I. & Bandyopadhyay, S. (2017). *Pet Bird Diseases and Care*. Springer Singapore. https://doi.org/10.1007/978-981-10-3674-3

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., Kartesz, J., Kenis, M., Kreft, H., Kühn, I., Lenzner, B., Liebhold, A., Mosena, A., Moser, D., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., van Kleunen, M., Walker, K., Weigelt, P., Yamanaka, T. & Essl, F. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications*, 8 (1), 14435. https://doi.org/10.1038/ncomms14435

Senar, J.C., Domènech, J., Arroyo, L., Torre, I. & Gordo, O. (2016). An evaluation of monk parakeet damage to crops in the metropolitan area of Barcelona. *Animal Biodiversity and Conservation*, 39, 141–145. https://doi.org/10.32800/abc.2016.39.0141

Simberloff, D. & Rejmanek, M. (eds) (2019). 100 of the World's Worst Invasive Alien Species: A Selection From The Global Invasive Species Database. In: *Encyclopedia of Biological Invasions*. University of California Press. 715–716. https://doi.org/10.1525/9780520948433-159

Strubbe, D. & Matthysen, E. (2009). Establishment success of invasive ring-necked and monk parakeets in Europe. *Journal of Biogeography*, 36 (12), 2264–2278. https://doi.org/10.1111/j.1365-2699.2009.02177.x

Stuart, K.C., Johnson, R.N., Major, R.E., Atsawawaranunt, K., Ewart, K.M., Rollins, L.A., Santure, A.W. & Whibley, A. (2024). The genome of a globally invasive passerine, the common myna, Acridotheres tristis. *DNA Research*, 31 (2), dsae005. https://doi.org/10.1093/dnares/dsae005

Sweet, F.S.T., Apfelbeck, B., Hanusch, M., Garland Monteagudo, C. & Weisser, W.W. (2022). Data from public and governmental databases show that a large proportion of the regional animal species pool occur in cities in Germany. *Journal of Urban Ecology*, 8 (1), juac002. https://doi.org/10.1093/jue/juac002

Taylor, B.W. & Irwin, R.E. (2004). Linking economic activities to the distribution of exotic plants. *Proceedings of the National Academy of Sciences*, 101 (51), 17725–17730. https://doi.org/10.1073/pnas.0405176101

Trevelline, B.K. & Kohl, K.D. (2022). The gut microbiome influences host diet selection behavior. *Proceedings of the National Academy of Sciences*, 119 (17), e2117537119. https://doi.org/10.1073/pnas.2117537119

Van Wettere, A.J. (2025). *Avian Chlamydiosis*. MSD Veterinary Manual. https://www.msdvetmanual.com/poultry/avian-chlamydiosis/avian-chlamydiosis [2025-10-22]

VetBact (2023a). *Escherichia coli*. https://www.vetbact.org/index.php?artid=68&vbsearchstring=Escherichia%20coli [2025-10-08]

VetBact (2023b). *Chlamydia abortus*. https://www.vetbact.org/index.php?artid=94&vbsearchstring=Chlamydia%20abortus [2025-12-01]

VetBact (2025). *Campylobacter upsaliensis*. https://www.vetbact.org/species/110 [2025-12-02]

Vourc'h, G., Moutou, F., Morand, S. & Jourdain, E. (2022). Defining Zoonoses. In: *Zoonoses the Ties that Bind Humans to Animals [Internet]*. Éditions Quae. https://www.ncbi.nlm.nih.gov/books/NBK596957/ [2025-10-02]

Walther, G.-R., Roques, A., Hulme, P.E., Sykes, M.T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukát, Z., Bugmann, H., Czúcz, B., Dauber, J., Hickler, T., Jarošík, V., Kenis, M., Klotz, S., Minchin, D., Moora, M., Nentwig, W., Ott, J., Panov, V.E., Reineking, B., Robinet, C., Semenchenko, V., Solarz, W., Thuiller, W., Vilà, M., Vohland, K. & Settele, J. (2009). Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution*, 24 (12), 686–693. https://doi.org/10.1016/j.tree.2009.06.008

WHO (2023). *One Health*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/one-health [2025-09-29]

Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., Brand, T. van den, Posit & PBC (2025). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics* (4.0.0). https://cran.r-project.org/web/packages/ggplot2/index.html [2025-10-27]

Williamson, M. (1996). *Biological Invasions*. 1. ed. (Population and Community Biology Series; 15). Chapman and Hall.

Xenoulis, P.G., Gray, P.L., Brightsmith, D., Palculict, B., Hoppes, S., Steiner, J.M., Tizard, I. & Suchodolski, J.S. (2010). Molecular characterization of the cloacal microbiota of wild and captive parrots. *Veterinary Microbiology*, 146 (3), 320–325. https://doi.org/10.1016/j.vetmic.2010.05.024

Yan, S., Zhang, Y., Huang, J., Liu, Y. & Li, S. (2024). Comparative study of gut microbiome in urban and rural Eurasian tree sparrows. *Animals*, 14 (23), 3497. https://doi.org/10.3390/ani14233497

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W. & Glöckner, F.O. (2014). The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research*, 42 [Database issue], D643–D648. https://doi.org/10.1093/nar/gkt1209

Popular science summary

Invasive species are present all around the world, causing economic or ecological damage, or putting human health at risk. In Europe, the rose-ringed and monk parakeets have established populations mainly in urban areas, due to both deliberate releases and accidental escapes from the exotic pet trade. Both species are agricultural pests in their natural environments and cause noise pollution as they gather in large numbers and vocalize.

Animals have bacteria and other microorganisms in their intestinal system, called gut microbiota. These microorganisms don't just aid in digesting food but are also necessary for the immune system and are able to affect behavior. The composition of an individual's gut microbiota is affected by many factors, including their diet. The gut microbiome does not only consist of helpful bacteria however but may also include microbes causing disease. The movement of living organisms may therefore increase the risk of spreading diseases. Alien species have the potential to introduce bacteria or other disease-causing agents that have not previously existed in the new environment, or to be better at spreading diseases that already exist in the new environment.

When animals are introduced to a new location, they need to adapt to the conditions in the new environment to be able to survive. One such adaptation is the adaptation of the gut microbiota. The parakeets have been introduced in many places around the world and have been able to grow thriving populations in areas with quite varied conditions, such as differences in temperature, seasonal length, and access to different animal and plant species. Parakeets, which have a naturally varied diet consisting of fruits, seeds, nuts, and sometimes insects or leaves need a diverse gut microbiome with different traits to optimize digestion of the foods they eat.

The aim of this study was to examine if the species also have the potential to be vectors of bacteria that cause sickness in humans or other animals, so-called zoonotic bacterial diseases, as well as to examine differences in gut bacteria composition depending on geographical location.

The study was carried out by collecting fecal samples in six European cities distributed along a north-south geographic gradient, as well as sampling three caged parakeets in southern Sweden. The samples from European cities were collected from the ground beneath congregation points of the different parakeet species, which was mostly in urban parks.

Multiple bacterial species with zoonotic potential were discovered in the study, which indicate both species as potential vectors of zoonotic disease in urban environments. Further studies need to be carried out to draw more conclusions on parakeets’ role in disease transmission. No significant conclusions about adaptations on a north-south gradient were possible based on the results of this study.

Appendix 1

Table 3: Sample codes and micro-barcoding sequences. Code explanation: PK/MM/US/DIRT = suspected P. krameri, M. monachus, unsure origin or dirt sample, followed by location, sample year and sample number. Example: First suspected P. krameri from Madrid,

Sample	***Code***	***Micro Barcoding***	***Sample index***
A1	PKSev251	I7 706 I5 1	CATGCCTA-GCGATCTA
A2	PKSev252	I7 706 I5 2	CATGCCTA-ATAGAGAG
A3	PKSev253	I7 706 I5 3	CATGCCTA-AGAGGATA
A4	PKSev254	I7 706 I5 4	CATGCCTA-TCTACTCT
A5	PKSev255	I7 706 I5 5	CATGCCTA-CTCCTTAC
A6	PKSev256	I7 706 I5 6	CATGCCTA-TATGCAGT
A7	PKSev257	I7 706 I5 7	CATGCCTA-TACTCCTT
A8	PKSev258	I7 706 I5 8	CATGCCTA-AGGCTTAG
A9	PKSev259	I7 707 I5 9	GTAGAGAG-AAAGCAAG
B1	PKSev2510	I7 707 I5 10	GTAGAGAG-TTGAAGCC
B2	PKSev2511	I7 707 I5 11	GTAGAGAG-TGCCGATT
B3	PKSev2512	I7 707 I5 12	GTAGAGAG-TTTGAACC
B4	PKSev2513	I7 707 I5 13	GTAGAGAG-GTCGAAGT
B5	MMVal251	I7 707 I5 14	GTAGAGAG-GCAAGTCA
B6	MMVal252	I7 707 I5 15	GTAGAGAG-AGGTCCTA
B7	MMVal253	I7 707 I5 16	GTAGAGAG-AAGTCTCC
B8	MMVal254	I7 708 I5 1	CCTCTCTG-GCGATCTA
B9	MMVal255	I7 708 I5 2	CCTCTCTG-ATAGAGAG
C1	MMVal256	I7 708 I5 3	CCTCTCTG-AGAGGATA

C2	MMVal257	I7 708 I5 4	CCTCTCTG-TCTACTCT
C3	MMVal258	I7 708 I5 5	CCTCTCTG-CTCCTTAC
C4	MMVal259	I7 708 I5 6	CCTCTCTG-TATGCAGT
C5	MMVal2510	I7 708 I5 7	CCTCTCTG-TACTCCTT
C6	MMVal2511	I7 708 I5 8	CCTCTCTG-AGGCTTAG
C7	MMVal2512	I7 709 I5 9	AGCGTAGC-AAAGCAAG
C8	MMVal2513	I7 709 I5 10	AGCGTAGC-TTGAAGCC
C9	MMVal2514	I7 709 I5 11	AGCGTAGC-TGCCGATT
D1	MMVal2515	I7 709 I5 12	AGCGTAGC-TTTGAACC
D2	USPad251	I7 709 I5 13	AGCGTAGC-GTCGAAGT
D3	USPad252	I7 709 I5 14	AGCGTAGC-GCAAGTCA
D4	USPad253	I7 709 I5 15	AGCGTAGC-AGGTCCTA
D5	USPad254	I7 709 I5 16	AGCGTAGC-AAGTCTCC
D6	USPad255	I7 710 I5 1	CAGCCTCG-GCGATCTA
D7	USPad256	I7 710 I5 2	CAGCCTCG-ATAGAGAG
D8	USPad257	I7 710 I5 3	CAGCCTCG-AGAGGATA
D9	DIRTPad258	I7 710 I5 4	CAGCCTCG-TCTACTCT
E1	USPad259	I7 710 I5 5	CAGCCTCG-CTCCTTAC
E2	USPad2510	I7 710 I5 6	CAGCCTCG-TATGCAGT
E3	PKAms251	I7 710 I5 7	CAGCCTCG-TACTCCTT
E4	PKAms252	I7 710 I5 8	CAGCCTCG-AGGCTTAG
E5	PKAms253	I7 711 I5 9	TGCCTCTT-AAAGCAAG
E6	PKAms254	I7 711 I5 10	TGCCTCTT-TTGAAGCC

E7	PKAms255	I7 711 I5 11	TGCCTCTT-TGCCGATT
E8	PKAms256	I7 711 I5 12	TGCCTCTT-TTTGAACC
E9	PKAms257	I7 711 I5 13	TGCCTCTT-GTCGAAGT
F1	PKAms258	I7 711 I5 14	TGCCTCTT-GCAAGTCA
F2	PKAms259	I7 711 I5 15	TGCCTCTT-AGGTCCTA
F3	PKAms2510	I7 711 I5 16	TGCCTCTT-AAGTCTCC
F4	MMBar251	I7 712 I5 1	TCCTCTAC-GCGATCTA
F5	MMBar252	I7 712 I5 2	TCCTCTAC-ATAGAGAG
F6	MMBar253	I7 712 I5 3	TCCTCTAC-AGAGGATA
F7	MMBar254	I7 712 I5 4	TCCTCTAC-TCTACTCT
F8	MMBar255	I7 712 I5 5	TCCTCTAC-CTCCTTAC
F9	MMBar256	I7 712 I5 6	TCCTCTAC-TATGCAGT
G1	MMBar257	I7 712 I5 7	TCCTCTAC-TACTCCTT
G2	MMBar258	I7 712 I5 8	TCCTCTAC-AGGCTTAG
G3	MMBar259	I7 736 I5 9	TGCTATGG-AAAGCAAG
G4	MMBar2510	I7 736 I5 10	TGCTATGG-TTGAAGCC
G5	MMBar2511	I7 736 I5 11	TGCTATGG-TGCCGATT
G6	MMMad251	I7 736 I5 12	TGCTATGG-TTTGAACC
G7	MMMad252	I7 736 I5 13	TGCTATGG-GTCGAAGT
G8	MMMad253	I7 736 I5 14	TGCTATGG-GCAAGTCA
G9	MMMad254	I7 736 I5 15	TGCTATGG-AGGTCCTA
H1	MMBar2512	I7 736 I5 16	TGCTATGG-AAGTCTCC
H2	MMBar2513	I7 738 I5 1	GCTATACC-GCGATCTA

H3	DIRTBar2514	I7 738 I5 2	GCTATACC-ATAGAGAG
H4	MMSwe251	I7 738 I5 3	GCTATACC-AGAGGATA
H5	MMSwe252	I7 738 I5 4	GCTATACC-TCTACTCT
H6	MMSwe253	I7 738 I5 5	GCTATACC-CTCCTTAC
H7	MMSwe254	I7 738 I5 6	GCTATACC-TATGCAGT
H8	MMSwe255	I7 738 I5 7	GCTATACC-TACTCCTT
H9	MMSwe256	I7 738 I5 8	GCTATACC-AGGCTTAG
Neg. control		I7 739 I5 11	GCATAACC-TGCCGATT

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