

Accepted Manuscript

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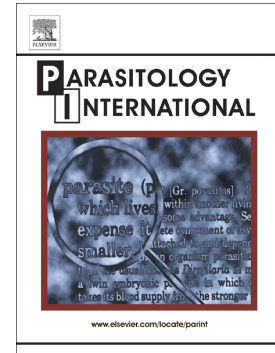
PII: S1383-5769(16)30557-8
DOI: doi: [10.1016/j.parint.2017.05.001](https://doi.org/10.1016/j.parint.2017.05.001)
Reference: PARINT 1675

To appear in: *Parasitology International*

Received date: 21 December 2016
Revised date: 2 May 2017
Accepted date: 4 May 2017

Please cite this article as: Marco Pezzi, Marilena Leis, Milvia Chicca, Lise Roy , Gamasoidosis by the special lineage L1 of *Dermanyssus gallinae* (Acarina: Dermanyssidae): A case of heavy infestation in a public place in Italy. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Parint(2017), doi: [10.1016/j.parint.2017.05.001](https://doi.org/10.1016/j.parint.2017.05.001)

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Gamasoidosis by the special lineage L1 of *Dermanyssus gallinae* (Acarina: Dermanyssidae): a case of heavy infestation in a public place in Italy

Marco Pezzi^{a, b,*}, Marilena Leis^{a, b}, Milvia Chicca^a, Lise Roy^c

^aDepartment of Life Sciences and Biotechnology, University of Ferrara, Via Luigi Borsari 46, 44121, Ferrara, Italy

^bLaboratory TekneHub, Tecnopole of University of Ferrara, Via Saragat 13, 44122, Ferrara, Italy

^cUMR 5175 CEFÉ, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier 3, EPHE - Campus du CNRS, 1919, route de Mende, 34293 Montpellier (France)

* Corresponding author at: Department of Life Sciences and Biotechnology, University of Ferrara,

Via Luigi Borsari 46, 44121 Ferrara, Italy. Tel.: ++39 532-455956; Fax: ++39 532-249761;

E-mail address: marco.pezzi@unife.it (M. Pezzi).

Running head: Gamasoidosis by *Dermanyssus gallinae* L1 in Italy

Highlights

- Mite infestations occurred in public conference halls in Northern Italy, causing gamasoidosis.
- Morpho-molecular analyses identified *Dermanyssus gallinae* lineage L1 as responsible for the infestation associated with rock doves.
- This was the first morpho-molecular characterisation of human infestation by the cryptic species *D. gallinae* L1.

ABSTRACT

Among Gamasina (Acari: Mesostigmata) mites, some dermanyssoid species are known to cause gamasoidosis, a human dermatitis characterized by papulosquamous eruptions and urticarian lesions. We describe a case of mite infestation which occurred in public conference halls in Ferrara (Italy), affecting four people who attended the place and showed signs of gamasoidosis. The mites were collected and characterized using scanning electron microscopy, light microscopy and mitochondrial DNA sequencing (Cytochrome c oxidase subunit I partial CDS). Based on morphological and molecular data, the species responsible for the infestation was identified as the special lineage L1 of the poultry red mite, *Dermanyssus gallinae* (De Geer) (Acarina: Dermanyssidae), a cryptic species known to be associated with pigeons. Rock doves, *Columba livia* Gmelin (Columbiformes: Columbidae) were roosting on the top of the public building, thus the mites probably gained access to the halls through small window openings. The present case report is the first one providing morpho-molecular identification of a *D. gallinae* cryptic species responsible of gamasoidosis in Italy.

Keywords: Poultry red mite; infestation; pigeon; ultrastructure; mtDNA; Co1

1. Introduction

Gamasoidosis is a zoonotic acariasis described as a human skin disease and caused by nonburrowing hematophagous dermanyssoid mites (Acari: Mesostigmata: Gamasina) that typically feed on birds and other animals. These mites belong to the families Dermanyssidae, hematophagous ectoparasites of rodents and birds, and Macronyssidae, ectoparasites preferentially of birds, mammals and reptiles [1]. Among Dermanyssidae species, *Dermanyssus gallinae* (De Geer) (Acari: Mesostigmata), the “poultry red mite”, has a high economic and veterinary relevance because of its worldwide occurrence and close association with domesticated birds [2,3]. Through its bite, *D. gallinae* causes debilitation by exsanguination and a high mortality rate in chicks and sometimes in hens [4]. In addition to dermatological disorders, *D. gallinae* has been implicated in the transmission of many agents responsible for serious diseases in animals and humans [5]. Moreover, in absence of the usual host it may also attack mammals, including humans, causing dermatitis of variable intensity, thus representing a significant medical and veterinary issue [6]. Based on morphological and molecular data, Roy et al. [7,8] and Roy and Buronfosse [9] have shown that *D. gallinae* is actually a species complex including at least two cryptic species (that is species that may not be distinguished from each other based on morphology alone, despite complete reproductive isolation). The host spectra of these cryptic species appear different: *D. gallinae* s. str. was recorded from poultry farms, as well as other bird species except pigeons, while *D. gallinae* special lineage L1 was recorded only from pigeons (both in France and in the USA). Here we describe a case of heavy infestation by *D. gallinae* L1 which occurred in two public conference halls in Ferrara (Italy). This affected four people attending one of the two conference halls and was accompanied by clear signs of gamasoidosis. The mite species and its avian origin were identified on the basis of ultrastructural observations and DNA sequencing.

2. Case report

In October 2015, some very small arthropods were found in a public conference hall in Ferrara (Italy) and one of them was delivered for identification to the Laboratory of Urban Ecology of the Department of Life Sciences and Biotechnology, University of Ferrara (Ferrara, Italy). Upon a first analysis under a stereomicroscope, the arthropod was tentatively identified as a mite. About 24 hours after the tentative identification, four people who sojourned for some hours in the same hall showed cutaneous signs of mite dermatosis with intense itching. The first one (a 20-year old girl) had signs in her left forearm (Fig. 1A and inset), the second one (a 23-year old girl) in her lower back (Fig. 1B), the third one (a 22-year old girl) on her left thigh (Fig. 1C) and the fourth one (a 20-year old boy) on his right hand. All four persons reported that for some days they had seen small “bugs” running on conference benches and on their clothes. An immediate inspection of the public hall confirmed a heavy infestation by mites there and in another one hall with similar architecture in the same building. About 40 live individuals were collected using fine brushes in both halls, stored in clean test tubes, then frozen (-20°C).

The morphological investigations were performed using scanning electron microscopy (SEM) and light microscopy. For SEM investigation, a total of six specimens (3 males and 3 females) were fixed, postfixed and dehydrated according to a previously published procedure [10]. The specimens were then critical point dried in a Balzers CPD 030 dryer (Leica Microsystems, Wetzlar, Germany), mounted on stubs and coated with gold-palladium in a Sputter Coated S150 Edwards (HHV Ltd, Crawley, UK). All SEM observations were conducted at the Electron Microscopy Centre of University of Ferrara on a Zeiss EVO 40 SEM (Zeiss, Milan, Italy).

The identification of the species was based on a careful morphological examination of the adult female (Figs. 2A, B), using the identification keys by Moss [11,12], Varma [13], Roy et al. [7] and Di Palma et al. [14].

The species was first identified as belonging to the *Dermanyssus gallinae* (De Geer) complex (Acarina: Dermanissidae) based on the following characters examined by SEM: genu of the second

and third leg pair each bearing two posterolateral setae (Figs. 2C, D); one posterolateral seta on each tibia of the fourth leg pair (Fig. 2E); a dorsal shield posteriorly tapering, with a truncate margin (Figs. 2A); two epigynal pores on the genitoventral shield (Fig. 2F); the anal shield roughly triangular and not oval, with the anal opening in the lower half and surrounded by three setae (Fig. 2G). The species identification was confirmed by examination at a Jeulin Optim (Evreux, France) light microscope which showed spatulate anterior lateral setae on the palp genu.

The species *D. gallinae* s. str. is commonly found on birds, especially chickens, *Gallus gallus domesticus* L. (Galliformes: Phasianidae). However, near the infested public hall, there were no chickens but several other bird species that could act as a host: *Columba livia* Gmelin (Columbiformes: Columbidae), *Passer domesticus* L. (Passeriformes: Passeridae), *Pica pica* L. (Passeriformes: Corvidae) and *Garrulus glandarius* L. (Passeriformes: Corvidae). The first species is known to be associated with *D. gallinae* L1, while the other ones may be associated with *D. gallinae* s. str. Morpho-molecular mite analyses were performed as follows: 3 adult female mites were prepared for molecular analyses, keeping their cuticle for species confirmation; cuticles were mounted in Hoyer's medium on glass microscope slides and observed using a light microscope. DNA template was obtained from the mites as in Roy et al. [15]. PCR amplification of an amplicon of Cytochrome c oxidase subunit I (Co1) was performed for each of the 3 different individuals [15]. PCR was conducted using a Perkin-Elmer PE 9700 Thermal cycler (Waltham, Massachusetts, USA) and both strands of final PCR products were sequenced by capillary electrophoresis by MWG Eurofins Operon sequencing laboratory (Ebersberg, Germany).

A single 643-bp DNA sequence was recovered from the three mite individuals (accession number: LT714694). The obtained sequence was submitted to nucleotide-nucleotide megaBLAST using the Basic Local Alignment Search Tool (BLAST) on [16] to identify the closest organisms based on similarity percentages to sequences in the Genbank. To refine the statement, a phylogenetic tree was then reconstructed: the evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model [17], as selected based on the AICc index using

MEGA6 [18]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with a superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5872)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 48.8844% sites).

Using the BLAST tool and considering only the matches with an E-value = 0.0 (39 entries) (Fig. 3), max. 1% sequence divergence was reported from sequences recorded as *D. gallinae* L1 in Roy et al. [8] and Roy and Buronfosse [9] and 9-12% from sequences recorded as *D. gallinae* s. str. Using homologous sequences from *D. carpathicus*, *D. hirundinis* and *D. apodis* as outgroups, the sequence from Ferrara mites clearly grouped with other L1 sequences as a sister clade to *D. gallinae* s. str.

The results confirmed that the mites collected in the public halls belonged to the *D. gallinae* cryptic species which to date has been found only on pigeons. On the roof of the building where the two public conference halls were located, a long-term colony of *C. livia* existed. We therefore concluded that the source of infestation was the *C. livia* colony.

Based on these results, a complete disinfestation of the public halls was immediately carried out using pyrethroid insecticides. The treatment was performed using deltamethrin (K-Othrine® Flow 25, Bayer, Milan, Italy) on floors and tetramethrin (Boxtar® Zapi, Padua, Italy) on furniture. The halls were fumigated using cyphenothrin (Dobol® Orma, Turin, Italy), sealed for 24 hours and then ventilated for other 24 hours before admitting the public. The *C. livia* colony area on the roof of the public building was carefully cleaned and the pre-existing anti-roosting spike strips were further extended. A program was established for monthly control of the halls for a year: no further infestation has been recorded.

All mite specimens, except the individuals collected for genetic analyses, were deposited at the Laboratory of Urban Ecology, Department of Life Science and Biotechnology, University of Ferrara, Ferrara (Italy).

3. Discussion

Dermanyssus gallinae (De Geer) (Acari: Mesostigmata), (“poultry red mite” or “red chicken mite”), is a blood-sucking, cosmopolitan ectoparasite [19,20]. Adult females feed mainly at night on the host for less than an hour every 2-4 days [3], but they may survive for up to nine months without feeding [20]. The female lays eggs 12-24 hours after feeding [21]; development includes five stages and the mites feed on blood from the protonymph stage onward [22]. *D. gallinae* s. str. was first associated with domestic chickens and other domestic and wild birds belonging to the orders Anseriformes, Columbiformes, Coraciiformes, Galliformes, Passeriformes and Stringiformes [23]. Subsequent morphomolecular analyses revealed there were cryptic species, so to a few misleading older morphology-based reports. The *D. gallinae* species found in Columbiformes, at least in pigeons, were not *D. gallinae* s. str. but *D. gallinae* L1 [9, 15]. Studies in the last decade have shown that most of the *Dermanyssus* species actually have more restricted host ranges than first believed [15], and some incorrect records exist because of high intraspecific and overlapping interspecific morphological variation [7,12]. *D. gallinae* L1 has a much more restricted host spectrum than *D. gallinae* s. str. and is associated only with pigeons to date (both in France and in USA) [8]. When an avian host is unavailable, the mites may attack mammals (including man) and their bites can be painful and irritating [13]. Two detailed descriptions of attacks by the *D. gallinae* complex on non-human mammals have been published, the first on a dog [24] and the second one on a horse [25]; in both cases poultry also existed near the mammals dwelled. Humans may be attacked by this species complex when they dwell or work in a place where usual avian hosts are nearby. In the reported cases of human infestations, the sources were wild or domestic birds, and/or their nests and roosting places. Among wild host species *C. livia* [15, 26-39] and *P. domesticus*

[40,41] have been reported and domestic ones include *Serinus canaria domestica* (Passeriformes: Fringillidae) [42] and *G. gallus domesticus* [36,41,43,44]. The known cues the *D. gallinae* complex uses for host location are thermal and mechanical [3]. In the reported case, the above cues, the outside temperature drop in early autumn and the recent abandoning of nests by fledglings may have resulted in a large population of mites moving to the public halls.

Infestations by *D. gallinae* in public spaces are usually associated with synanthropic birds, mostly pigeons and their nests and roosts. Several cases of nosocomial infestations ascribable to *C. livia* proximity have been reported for this mite, which attacked patients [26,29,32,40], visiting relatives [27] and health professionals [28]. Similar infestations in public spaces were reported in an army residence and university offices in Spain [35]. Only one case of infestation by the *D. gallinae* complex in a public place (a school) was previously reported in Italy [31].

Based on all the above literature and the results of this study, we suggest that between the two *D. gallinae* cryptic species, *D. gallinae* L1, which is associated to pigeons, is the most common synanthropic *Dermanyssus* species in urban contexts, and thus could represent a potential risk for human health. However, the two species of the *D. gallinae* complex, *D. gallinae* L1 and *D. gallinae* s. str., are not distinguishable based solely on morphological characters and their specific diagnostics requires DNA analysis [9]. Since the reproductive isolation was proved by both mitochondrial and nuclear DNA phylogenetic analyses, to discriminate between the two cryptic species, a single sequence, such as the Co1 mitochondrial region [7-9,15], is suitable for sequence-based diagnostics. Diagnostic PCR primers could be developed for that region to provide a correct identification of the agent responsible for gamasoidosis using a simple PCR-electrophoresis method, thus avoiding DNA sequencing and avoiding time-consuming and expensive diagnostics. The clinical symptoms of infestation by the *D. gallinae* complex include erythematous rashes often excoriated because of pruritus, small blisters, urticarial plaques and erythema [1,45]. The infestation may also affect the scalp, with the onset of dermatitis and intense pruritus [36,44]. Two cases of occupational otitis externa were reported in poultry workers [43].

This species complex has been associated with zoonotic diseases of bacterial origin, among them *Pasteurella multocida* (Lehmann & Neumann) (Pasteurellales: Pasteurellaceae) and *Coxiella burnetii* (Legionellales: Coxiellaceae) and viral agents of Fowlpox, Eastern equine encephalitis, Western equine encephalitis and Venezuelan equine encephalitis [22].

From a medical point of view, the infestation by *D. gallinae* complex may easily be eradicated by careful personal hygiene and in most cases no medical treatment is required. When bites are painful and itching, rapid relief may be obtained by topical applications of anti-histamines and corticosteroid drugs [37]. In the case of scalp infestation, treatments with 1% permethrin shampoo are able to remove the mites [36]. The relative ease in removing mites from the human body and scalp is probably due to the fact that parasites stay on host only for the time required for the blood meal. This behaviour is completely different from that of another haematophagous ectoparasite, *Ornithonyssus sylviarum* Canestrini & Fanzago (Mesostigmata: Macronyssidae) which carries out its entire life cycle on the host [1]. For an effective eradication of mites from infested places, a correct identification of the pest species may be highly useful to target the right source of infestation. In our case the identification of the cryptic species *D. gallinae* L1 was possible by integrating morphological observations with molecular analyses of mitochondrial DNA sequences: these data allowed us to identify with precision the avian source of infestation, *C. livia*, among all bird species roosting on the public hall roof. Once one has identified the avian source of infestation, it is necessary to discourage the birds from roosting on buildings by removing nests and waste materials, carefully sealing crevices and cracks, and installing suitable deterrent systems; treatments with insecticides and careful vacuum cleaning of the places infested must also be performed [37]. The increasing reports of mite attacks on non-avian hosts are best addressed by close cooperation among physicians, veterinarians and entomologists.

Although another case of infestation by *D. gallinae* complex has been previously reported in a school in Italy, our case is the first one identifying one of the two species of the complex, *D. gallinae* L1, as responsible of the infestation, based on both ultrastructural and molecular data.

These analyses allowed to precisely identify the avian host species, providing pest controllers with key information for targeted and effective treatments.

Conflict of interest

The authors declare no conflict of interest related this work.

Acknowledgements

Funds supporting this study were provided by CTB-PROG-ZT-2016, AUSL Cesena, assigned to the Department of Life Sciences and Biotechnology, University of Ferrara (Ferrara, Italy). The manuscript is dedicated to the loving memory of Mrs. Delfina Costa.

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Figure captions

Fig. 1. Clinical aspect of gamasoidosis caused by *Dermanyssus gallinae* L1. (A) Grouped papules on left forearm of the first patient. Inlay: other papules on left forearm of the same patient. (B) Grouped papules on lower back of the second patient. (C) Grouped papules on left thigh of the third patient.

Fig. 2. Scanning electron micrographs of female of *D. gallinae* L1. (A) Habitus in dorsal view. The arrows indicate the shoulders of dorsal shield. (B) Habitus in ventral view. The rectangle outlines the region enlarged in Fig. 2F. The dashed square outlines the region enlarged in Fig. 2G. (C) Right genu of the second leg pair. The arrows indicate the posterolateral setae. Inlay: overview of the anterolateral part of the right dorsal side. (D) Right genu of the third leg pair. The arrows indicate the posterolateral setae. (E) Right tibia of the fourth leg pair. The arrow indicates the posterolateral seta. (F) Detail of Fig. 2B showing the left epigynal pore (arrow) on the genitoventral shield. (G) Detail of Fig. 2B showing the anal shield. The arrows indicate the three anal setae. Terminology follows Moss [11,12] and Di Palma [14]. AS, anal shield; DS, dorsal shield; G, genu; GV, genitoventral shield; L1-4, leg; PA, palp; T, tibia.

Fig. 3. Molecular Phylogenetic analysis using the Maximum Likelihood method of partial Co1 coding gene from the “Pezzi” mite isolate, as well as other isolates belonging to both *D. gallinae* s. str. and *D. gallinae* L1 (collected from the Genbank following BLAST analysis of the unique Co1 sequence obtained from Ferrara mites; 39 entries with E-value = 0.0). Isolates from three non *gallinae* species of *Dermanyssus* were inserted as outgroups (the analysis involved 49 nucleotide sequences altogether). The tree with the highest log likelihood (-1965.3640) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 519 positions in the

final dataset. Genbank accession numbers are followed by isolate or clone names in parentheses.

The unique Co1 sequence from Ferrara mites, labelled “**Pezzi isolate**” (bold underlined indicated by a grey triangle), has the accession number: LT714694.

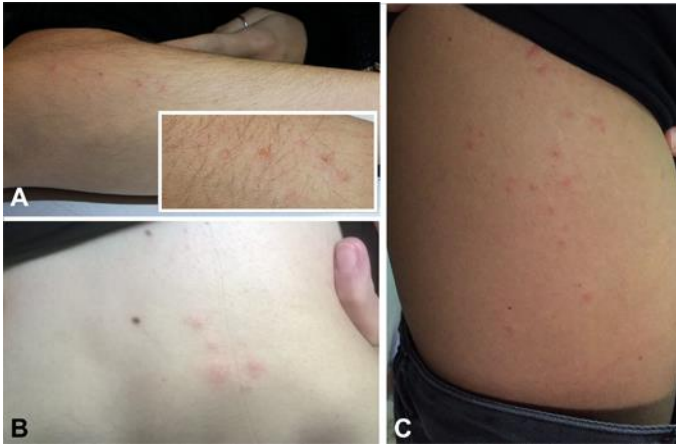


Fig. 1

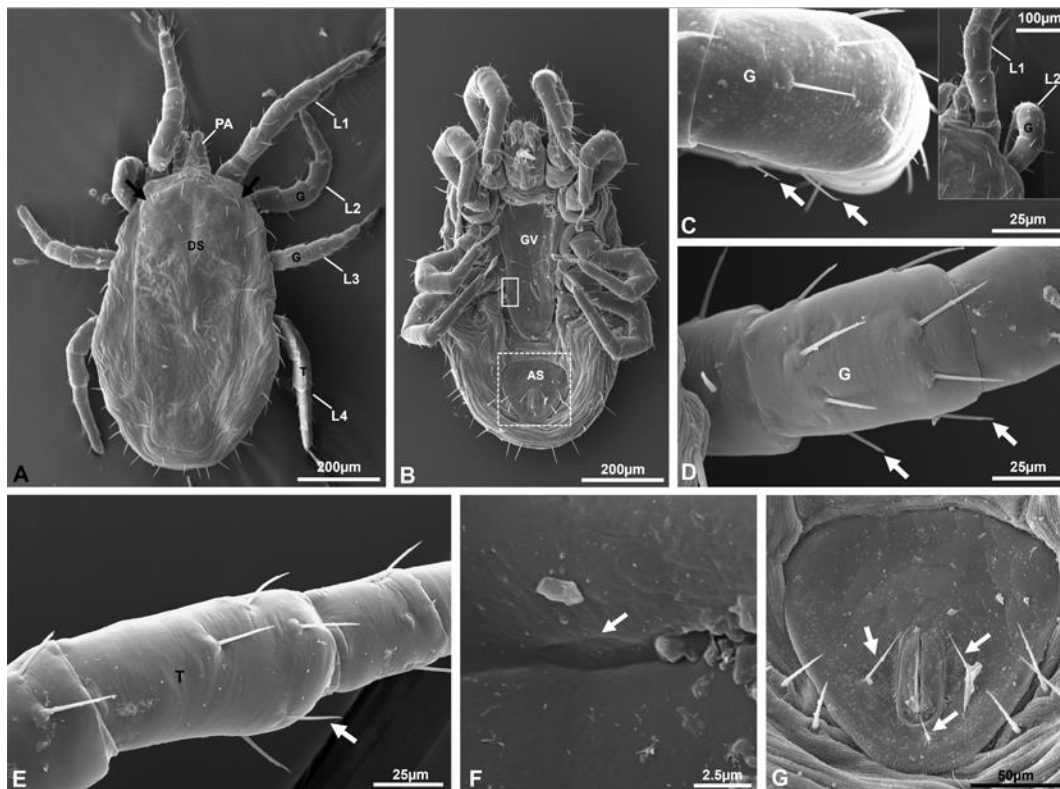


Fig. 2

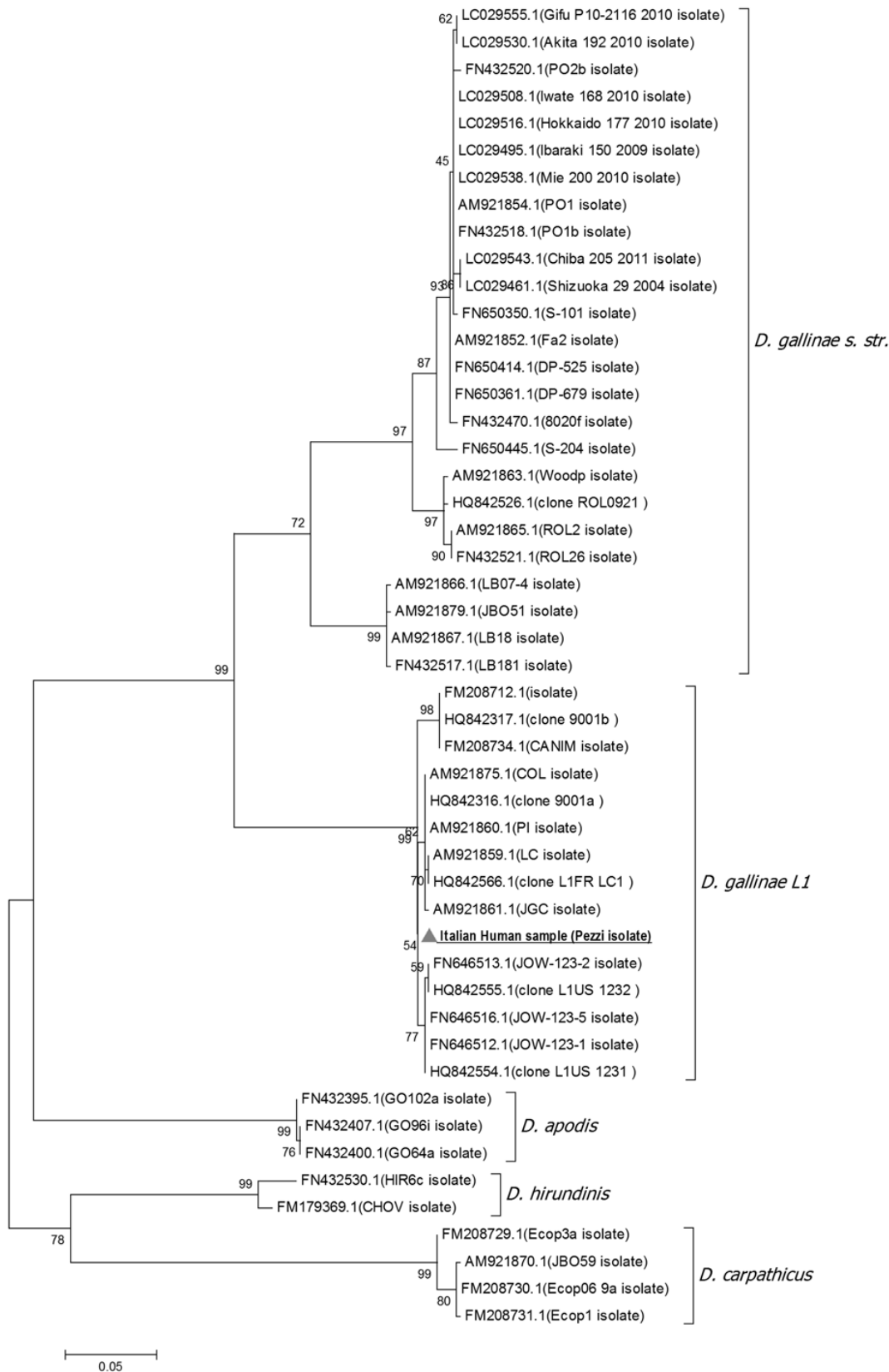


Fig. 3