

# *Neottialges (Caloenectes) vulturis* (Dubinin, 1956) (Acari: Hypoderatidae) from the Eurasian griffon vulture (*Gyps fulvus*) in Italy: first record in Europe, redescription and pathological changes in the host

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## ABSTRACT

Deutonymphs of the astigmatan mite *Neottialges (Caloenectes) vulturis* (Dubinin, 1956) comb. n. (Astigmata: Hypoderatidae) were found during the anatomo-histopathological study of a Eurasian griffon vulture, *Gyps fulvus*, found dead in Central Italy. After skinning, numerous cystic formations were visible in the subcutaneous tissue of the vulture, each containing a parasitic mite. Microscopically, subcutaneous tissue showed large areas of severe granulomatous inflammation. To our knowledge, this is the first record of this poorly known hypoderatid mite in Europe, about five thousand kilometers west from the type locality in Kirghizstan. The deutonymphal stage of *N. (C.) vulturis* is redescribed following modern standards of taxonomic description for astigmatan mites. This mite, previously unassigned to a subgenus, is now placed in the subgenus *Caloenectes* Fain, 1966.

**Keywords** *Neottialges vulturis*, mites, parasites, *Gyps fulvus*, Italy

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## Introduction

Mites of the family Hypoderatidae are mainly associated with birds and rarely occur in rodents. At the deutonymphal stage (also named hypopus in Astigmata), these mites are subcutaneous or visceral tissue parasites, whereas the other life stages (larva, protonymph, tritonymph and imago) are commensals inhabiting the nests of corresponding vertebrate hosts (Fain 1967; Fain and Bafort 1967; OConnor 1982 and 1985; Wurst and Havelka 1997; Krantz and Walter 2009). An unusual way of feeding by hypopi in the host tissues using a modified genital papillae was recently revealed by Alberti and co-authors (2016) with an example in *Neottialges evansi* Fain, 1966 parasitizing cormorants.

This family currently includes over 80 species in 21 genera. The greatest majority of hypoderatid species are known only from deutonymphs, while adults and tritonymphs are described for only 20 species (Mironov and Kivganov 2010; Mironov and OConnor

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2013). *Neottialges vulturis* (Dubinin, 1956) was incompletely described by Dubinin (1956) from two vulture species, *Gyps fulvus* (Hablizl) (type host) and *Gyps himalayensis* Hume (Accipitriformes: Accipitridae), from Asian republics of the former USSR. Since that time, the mite has never been recollected. In the present work, we provide a modern redescription of the congested deutonymph of this species and describe pathological changes provoked by the presence of numerous mites in the subcutaneous tissues of a Eurasian griffon vulture, *G. fulvus*, found dead in Central Italy.

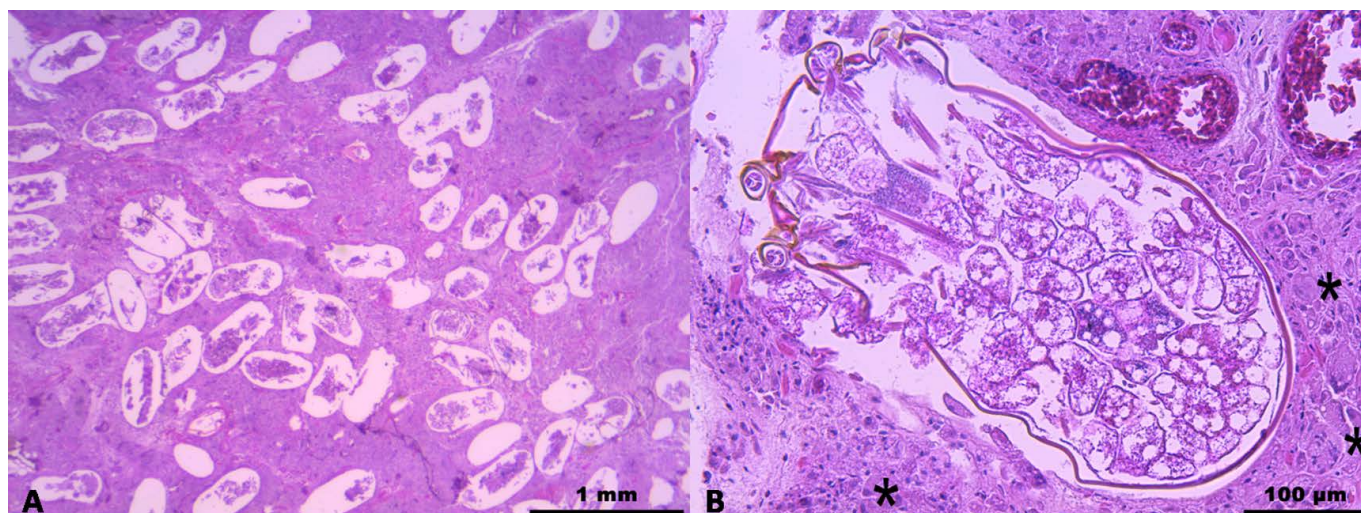
The Eurasian griffon vulture is a protected species in Italy and inhabits 5 distinct areas from the eastern Alps to the southern Apennines, Sardinia and Sicily. These populations mainly originate from reintroduction and restocking efforts, and, due to persistently high human-induced mortality and a low number of breeding individuals, the species is regarded as critically endangered at a national scale (Rondinini *et al.* 2013). Although the griffon vulture was once likely widespread throughout peninsular Italy, the last account of its occurrence along the central Apennines (specifically dealing with the Marche region) dates back to the XVI century (Pandolfi and Zanazzo 1993), after which time it became extinct. Between 1994 and 2002, 93 vultures from Spain were re-introduced in the central Apennines (Monte Velino reserve, Abruzzo region), where they successfully settled (Potena *et al.* 2009), establishing five reproductive colonies, from 1997 to 2012 (Altea *et al.* 2016). At present, up to 45 nesting pairs are estimated, and resident adults range across ca. 7,000 km<sup>2</sup> (Altea *et al.* 2013).

## Materials and methods

**Case report.** On the 6<sup>th</sup> of March 2015, an adult Eurasian griffon vulture was found dead in the Borgorose Municipality, Rieti Province, Lazio Region, Central Italy. The bird, discovered by local people, was recovered by the Forest Service near a paved road at the base of a concrete pole (supporting a power line) the day after a severe windstorm occurred in the area. From the data reported on the ring, it was possible to ascertain that the individual was born in Spain in 2008. It had been recovered in bad condition in Saldaña municipality (Spain), where it was ringed (metal and yellow-coloured ring, code CV8) and released from the Burgos rehabilitation centre on December 19<sup>th</sup> 2008. This vulture was photographed for the first time in Italy on December 2011, at the Monte Velino Reserve feeding point (Forest Service, unpublished data). It was then re-sighted several times in this area in December 2011 and January 2012. Subsequently, it was no longer recorded until March 2013, whereupon it became a regular monthly visitor at the feeding point, until December 2014. During management and research activities carried out by the Forest Service, it was captured on July 28<sup>th</sup> 2014. At this time, it weighed 8.05 kg and was sexed as a male by molecular markers from plucked feathers (Garofalo *et al.* 2016).

**Anatomo-histopathological study.** At necropsy the vulture weighed 9.4 kg and appeared in good health status. It was possible to ascertain that the bird was dead as a result of an impact. Large haematomas were detected in subcutis and muscles of neck, head and chest and haemorrhagic effusions were present in splanchnic cavity between the heart and lungs. At skinning, the subcutaneous tissue was diffusely thickened and grainy in appearance. Very numerous whitish, translucent, 0.5-1 mm nodules, covered almost the whole surface of subcutis and, to a lesser extent, the superficial muscles of chest, back and legs. For histological examinations, samples of subcutis were fixed in 10% buffered formalin, embedded in paraffin, cut at 5 µm and then stained with hematoxylin and eosin.

Microscopically, the subcutaneous tissue showed large areas of severe granulomatous inflammation, with many macrophages with a foamy appearance due to the phagocytosis of lipidic debris, probably of parasitic origin, and, to a lesser extent, multinucleated giant cells. Numerous cystic formations (Fig. 1a), each containing a parasitic mite, were seen (Fig. 1b). Foci of lymphocytes and plasma cells were occasionally observed, sometimes surrounding parasitic debris. A similar pathological picture was previously described by Schwan and Sileo (1978) in a *Phalacrocorax carbo* (Linnaeus) parasitized by the mite *Neottialges evansi* Fain 1966. By



**Figure 1** *Neottialges (Caloenectes) vulturis* in subcutaneous tissues of *Gyps fulvus*: A – numerous cysts containing mites, surrounded by a granulomatous reaction. B – cyst with a sectioned parasitic mite. Note the numerous foamy macrophages \* surrounding the cyst. Hematoxylin and eosin stain.

gently crushing some of the cysts with a needle under the stereo-microscope, it was possible to isolate the mites.

**Taxonomic study.** The redescription and measurement techniques are given according to the modern standard used for hypoderatid mites (Wurst and Havelka 1997; Mironov and Kivganov 2010; Mironov and OConnor 2013). The general terms follow OConnor (1985), the idiosomal chaetotaxy follows Griffiths *et al.* (1990) and the leg chaetotaxy is that of Grandjean (1939) and Griffiths (1964). All measurements are in micrometers (µm). The material used for taxonomic study is deposited in the Zoological institute of the Russian Academy of Sciences (Saint Petersburg, Russia).

## Systematics

### Family Hypoderatidae Murray, 1877

#### Genus *Neottialges* Fain, 1966

The genus *Neottialges* was established by Fain (1966) and originally included six species; at present it incorporates 28 species and is the most specious within the family (Mironov and Kivganov 2010). Within this genus, Fain (1966, 1967) originally recognized three subgenera, *Caloenectes* Fain, 1966, *Neottialges* s. str., and *Pelecanectes* Fain, 1966. Further, Fain and Lukoschus (1986) established two more subgenera, *Ardeidectes* Fain and Lukoschus, 1986 and *Heronidectes* Fain and Lukoschus, 1986. Representatives of this genus are presently known from birds of the orders Accipitriformes, Ciconiiformes, Charadriiformes, Columbiformes, Musophagiformes, Falconiformes and Pelecaniformes (Fain 1967; Fain and Lukoschus 1986; Mironov and Kivganov 2010). In the world revision of hypoderatids parasitizing birds, Fain (1967) did not assign *Neottialges vulturis* to any subgenus, because he was unable to re-examine any specimens of this mite. Based on the recollected material we examined in the present study, we place this species in the subgenus *Caloenectes* (see remark below).

***Neottialges (Caloenectes) vulturis* (Dubinin, 1956) n. comb.  
(Figures 2–4)**

*Gabucinia vulturis* Dubinin, 1956: 213, fig. 83-86.

*Neottialges vulturis*, Fain 1967: 107.

*Gypsodectes vulturis*, Fain 1984: 271.

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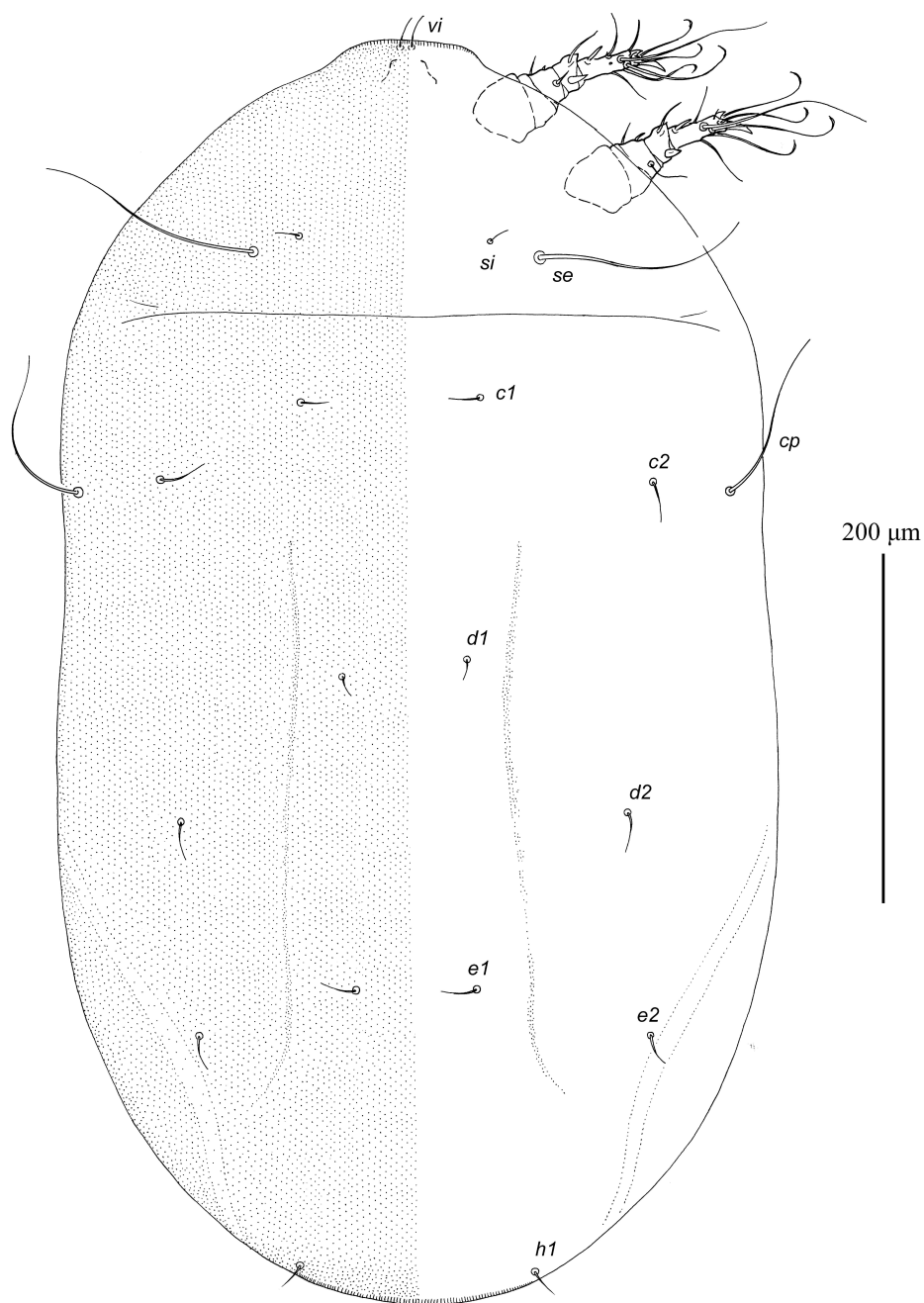
Material examined — 15 deutonymphs from *Gyps fulvus* (Accipitriformes: Accipitridae), Italy, Central Italy, Rieti Province, Borgorose Municipality, 6 March 2015, mite collector F. Tancredi.

Description — Deutonymph (range for 10 measured specimens). Idiosoma widely ovate, with very short and wide rostral extension, length 660 – 740, greatest width 300 – 400. Most surface of dorsal and ventral cuticle of idiosoma monotonously and roughly punctuated, borders of any shields indistinct (Figure 2). Sejugal furrow dorsally pronounced. Length of hysterosoma 510 – 570, central part with a pair of narrow longitudinal ridges, posterolateral parts with oblique poorly sclerotized grooves. Vertical setae *vi* filiform, situated on rostral extension submarginally, 12 – 15 long. Scapular setae *si* situated slightly anterior to level of setae *se*; distances between scapular setae: *se:se* 145 – 170, *si:si* 95 – 115, *si:se* 8 – 15; length of setae: *se* 145 – 170, *si* 10 – 12. Hysterosomal setae filiform; only three pairs of hysteronotal setae (*cp*, *c3*, and *h3*) distinctly long and comparable in length to scapular setae *se*; remaining setae of dorsal side and posterior end of hysterosoma not exceeding 20; length of long setae: *cp* 90 – 100, *c3* 85 – 95, *h3* 105 – 150. All cupules (*ia*, *im*, *ip* and *ih*) indistinct.

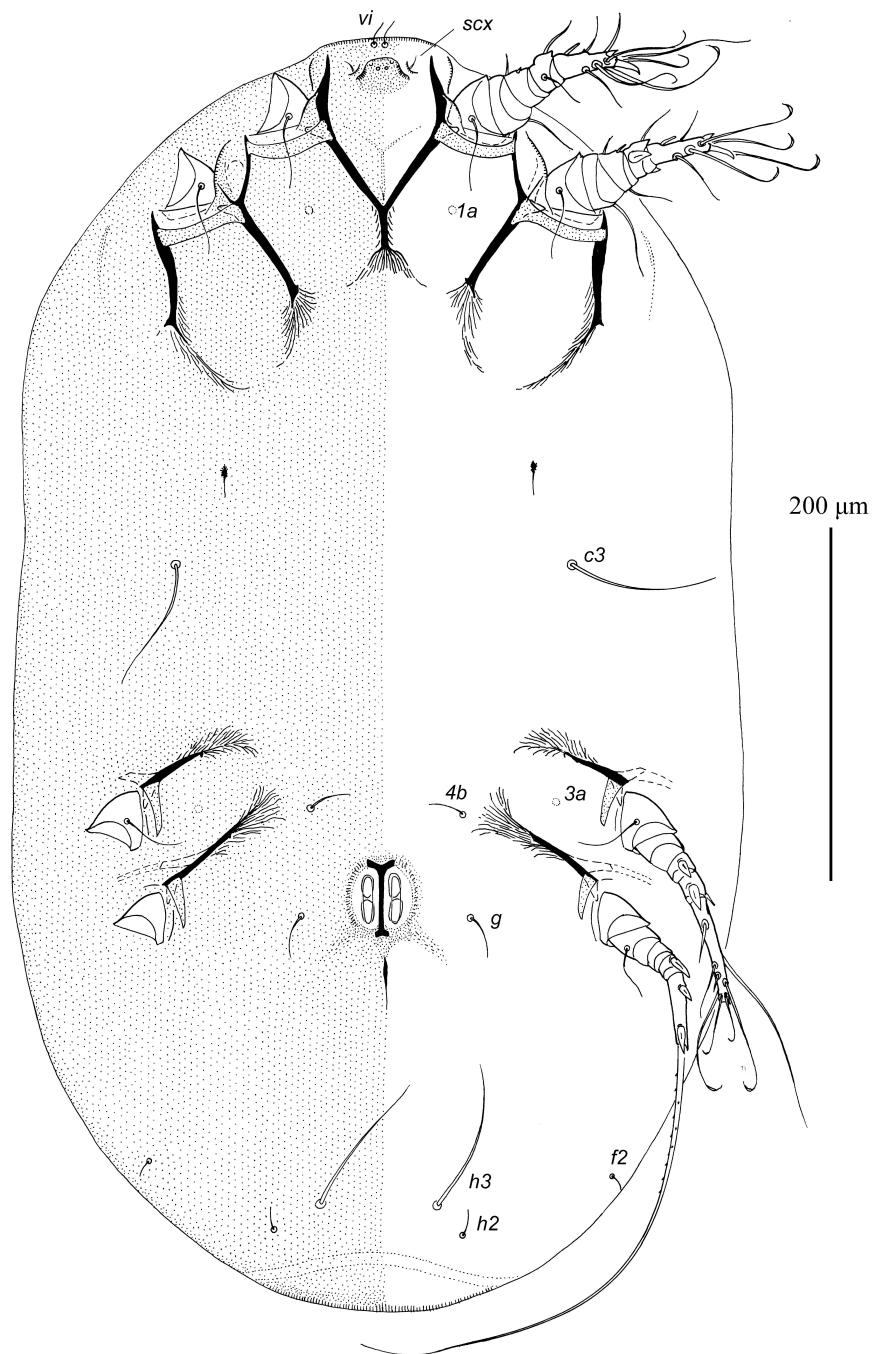
Gnathosoma reduced to small trapezium-shaped sclerite 15 – 20 long and 27 – 32 wide at base, with two pairs of rudimentary setae represented by alveoli (Figures 3, 4A). Supracoxal setae *scx* situated close to lateral margins of gnathosomal plate in small invaginations. Sternum 22 – 25 long, approximately half the length of free parts of epimerites I. Coxal fields I–IV open. Inner tips of all epimerites free, with irregular pennate striation on body cuticle. Bases of all trochanters I–IV flanked by narrow sclerotized bands. Coxal setae *1a* and *3a* rudimentary, represented by alveoli. Genital field ovate, situated at level of trochanters IV well outlined. Genital sclerite well developed, narrow, one-pieced; anterior and posterior ends T-shaped, width of anterior end nearly twice as wide as posterior end; length of sclerite 34 – 36, width of anterior end 10 – 12, width of posterior end 5 – 7 (Figure 4B). Genital papillae ovate, large, anterior and posterior pairs similar in size, length 11 – 12. Anal opening rudimentary, situated near posterior end of genital sclerite. Coxal setae *4b* 30 – 35 situated at midlevel of coxal fields III, genital setae *g* 33 – 37 long.

Leg segments normally developed, tarsi I, II subequal to total length of corresponding tibiae and genua, tarsi III, IV nearly 2 times longer than corresponding tibiae and genua. Length of legs excluding trochanters: I 80 – 85, II 82 – 86, III 115 – 120, IV 85 – 90; length of tarsi: I 30 – 33, II 30 – 34, III 58 – 62, IV 42 – 45. Tarsus I with setae *ba*, *d*, *wa* long filiform; setae *la*, *ra*, *p*, and *q* long filiform with foliate distal tips, seta *aa*, represented by alveolus, seta *e* short filiform, seta *f* spine-like, solenidion  $\omega 1$  distinctly punctated and slightly attenuate apically; solenidion  $\omega 3$  elongate, situated apically; famulus  $\epsilon$  at midlevel of tarsus, at same level as setae *ba* and *wa* (Figure 4C). Tarsus II similar in structure to tarsus I (except for absence of seta *aa* and famulus) (Figure 4D). Tarsus III strongly elongate and straight, with small bidentate apical spine, with 8 setae: setae *d* long filiform, setae *e*, *f*, *p*, *q*, and *r* filiform with foliate apices, seta *s* short spiculiform, seta *w* strongly thickened basally, with filiform apex (Figure 4E). Tarsus IV with small apical spine and with 4 setae of uncertain homology: apical seta *d* represented by macrosetae nearly 3 times longer than leg IV and with small sparse barbs in basal part; two spine-like setae *w*, *r* situated basally, and thin spine-like setae *s* situated subapically (Figures 4F). Empodial claws of tarsi I, II slightly longer than half-length of corresponding tarsi, acute and slightly curved apically, 15 – 17 and 14 – 16 long, respectively; empodial claw of tarsus

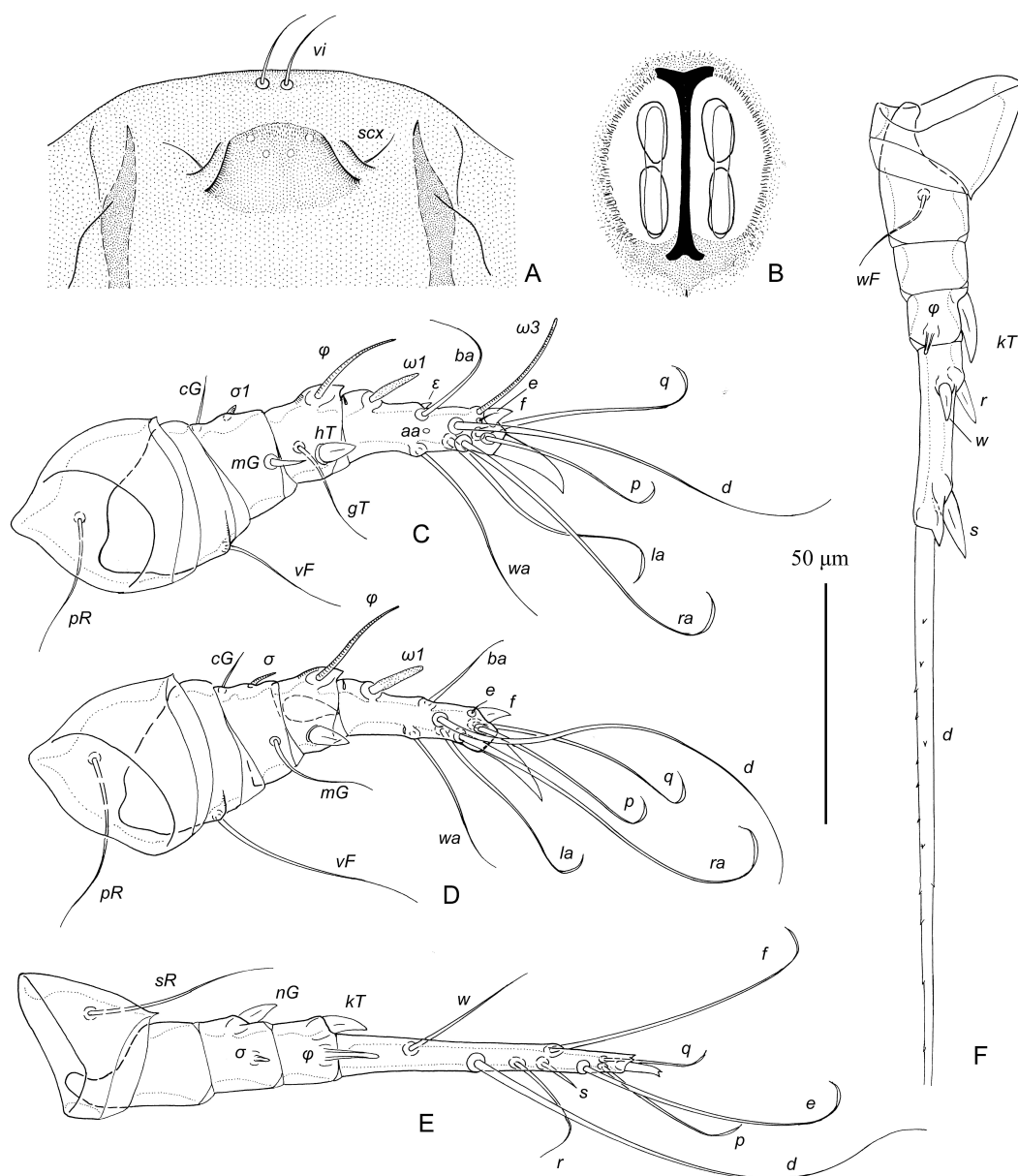




**Figure 2** *Neottialges (Caloenectes) vulturis*, dorsal view of deutonymph.



**Figure 3** *Neottialges (Caloenectes) vulturis*, ventral view of deutonymph.



**Figure 4** *Neottialges (Caloenectes) vulturis*, details of deutonymph: A – anterior end of propodosoma, ventral view; B – genital field; C–F – legs I–IV, respectively, dorsal view.

III bidentate apically and slightly curved, 7 – 9 long. Tibia I with longitudinal dorsal crest, seta *gT* long filiform, seta *hT* thick spine-like, solenidion  $\varphi$  about 1.5 times longer than this segment. Tibia II similar to tibia I except both setae *gT* and *hT* spine-like. Tibia III: with seta *kT* thick spine-like, solenidion  $\varphi$  short, attenuate to apex, similar in length to this segment, with empodial claw bidentate. Tibia IV with seta *kT* thick spine-like and solenidion  $\varphi$  half the length of this segment. Genu I with seta *mG* thick spiculiform, seta *cG* thin spiculiform, solenidion  $\sigma 1$  a very short and blunt spine. Genu II with seta *mG* filiform, seta *cG* as in tarsus I, and solenidion  $\sigma$  spiculiform. Genu III with seta *nG* thick spine-like, and with solenidion  $\sigma$  small spiniform. Femora I, II, IV with seta *vF* filiform, shorter than corresponding legs. Setae

*pR* of trochanters I, II subequal in length to corresponding tarsi, seta *sR* of trochanter III shorter than corresponding tarsus. Leg chaetotaxy (solenidia in brackets): tarsi 10(3)-9(1)-8-4, tibiae 2(1)-2(1)-1(1)-1(1), genua 2(1)-2(1)-1(1)-0, femora 1-1-0-1, trochanters 1-1-1-0.

Remarks — Dubinin (1956) described this hypoderatid species from a deutonymph, thinking it was part of the feather mite genus *Gabucinina* Oudemans, 1905 (Gabuciniidae), since he erroneously suggested that feather mites (Astigmata: Analgoidea and Pterolichoidea) can have a deutonymph in their life cycle. In the world revision of hypoderatids, Fain (1967) provisionally placed this mite species in the genus *Neottialges* Fain, 1966, because he had no specimens to examine. In the present paper, we confirm this mite belongs to the genus *Neottialges* and place it in the subgenus *Caloenectes* Fain, 1966 based on the following subgeneric characteristics observed in this species: the cuticle of the entire idiosoma is strongly and monotonously sclerotized and dorsomedian setae *dl*, *el* and *hl* are very short. Among the seven previously known species of this subgenus (Fain 1966, 1967 and 1973; Pence 1973; Fain and Lawrence 1986), *N. (C.) vulturis* is most similar to *N. (C.) kutzeri* Fain, 1967 in having laterocoxal setae *scx* situated closely to the gnathosomal plate, coxal setae *la* and *3a* rudimentary, and oblique poorly sclerotized grooves in posterolateral parts of idiosoma. Nevertheless, deutonymphs of *N. (C.) vulturis* clearly differ from those of the latter species by the following features: coxal fields II and III are open (vs. closed), the genital sclerite is well developed (vs. absent), the rudimentary anal opening is close to the genital fields (vs. situated far posteriorly), setae *c1* and *c2* are short, not exceeding 20 µm (vs. subequal to macrosetae *cp*), and setae *4a* and *g* are 30 – 40 µm long (vs. about 80 µm). The presence of the well sclerotized genital sclerite and the position of the anal opening close to the genital field differentiate this species from all previously known species of the subgenus *Caloenectes*.

It is necessary to note that Fain (1984) established a new hypoderatid genus *Gypsodectes* Fain, 1984 with the type species, *Gypsodectes verrucosus* Fain, 1984, described from a single female found in the nest of *Gyps coprotheres* (Forster JR) in South Africa. Based on this finding, he suggested that *Neottialges vulturis*, known only from deutonymphs, also belongs to the genus *Gypsodectes*. However, he did not have any real proof, such as pharate specimens (deutonymph in exuvium of protonymph or tritonymph in exuvium of deutonymph), to show that *N. vulturis* hypopi correspond to adult mites of the genus *Gypsodectes*. Deutonymphs in Astigmata are so morphologically different from all other stages of hypoderatids that it is practically impossible to guess their correspondence without pharate specimens, even when hypopi and other stages are found in the same nest. Indeed, there are a number of examples where birds simultaneously house several different hypoderatid species, usually belonging to different genera (Fain 1967; Fain and Lukoschus 1986; Mironov and Kivganov 2010; Mironov and OConnor 2013). Therefore, we retain the hypoderatid mite considered herein in the genus *Neottialges*.

## Discussion

This is the first report of *N. vulturis* after its original description over 60 years ago (Dubinin 1956). Interestingly, this report is located about five thousand kilometers west of the type locality in Kirghizstan and for the first time in Europe. The examined vulture was born in Spain and quite possibly it arrived in Central Italy already infested, as the primary portion of hypoderatid deutonymph infections usually comes from parents via the nest, where the remaining part of life cycle of these mites takes place, and where the next generation of deutonymphs appears. Therefore, the actual distribution of *N. vulturis* should likely be extended two thousand kilometers westward. The geographic distribution of this mite could overlap completely with that of the Eurasian griffon vulture, extending from Portugal, Morocco and Algeria in the west to Asian republics of the former USSR and India in the east, and from Yemen and Ethiopia in the south to Kazakhstan in the north. It could even cover the range of



the whole genus *Gyps*, extending across areas of Europe, Asia and Africa, since it was also recorded from *Gyps himalayensis*.

Notwithstanding this theoretically large geographical distribution, these mites can be considered as neglected parasites. Probably because of nature of the host and the seemingly low pathological effect they elicit, there are few reports and data quantifying the pathological impact of these mites on their hosts. In the present case, the number of encysted mites, involving both subcutaneous and muscular tissues, was very high, in the order of thousands, and a marked inflammatory reaction was detectable. It is difficult to ascertain if this pathological picture had some effect on host fitness and survival, but a reduced functionality of the skin and animal suffering cannot be ruled out, despite of the apparent good health status revealed at necropsy. Nevertheless, Schwan and Sileo (1978) described similar pathological findings in a *Phalacrocorax carbo* parasitized by *Neottialges evansi* deutonymphs, reporting no apparent clinical discomfort in the bird.

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