

Towards resolving the double classification in *Erythraeus* (Actinotrichida: Erythraeidae): matching larvae with adults using 28S sequence data and experimental rearing

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Received: 28 November 2015 / Accepted: 24 April 2016 / Published online: 10 May 2016
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Abstract The taxonomy of free-living adults and heteromorphic parasitic larvae of Parasitengona mites has in the past been treated independently resulting in a double classification. Correct linkage of names still remains unknown for many species. A holistic understanding of species is imperative for understanding their role in ecosystems. This is particularly true for groups like parasitengone mites with a radically altered lifestyle during development—parasitic to predatory. Here, we infer linkages of three nominal species of *Erythraeus*, using matching with 28S DNA sequence data from field-collected specimens and through laboratory rearing. The general mixed Yule coalescent method (GMYC) was used to explicitly test if field-collected specimens representing heteromorphic life instars were conspecific. The field-collected larvae were allocated to adults of *Erythraeus cinereus* and *Erythraeus regalis*, respectively. Laboratory rearing of the same two species confirmed the matching done by DNA. Rearing was also successful for *Erythraeus phalangoides* after eggs were treated to an imitated winter diapause. This integrative

taxonomic approach of molecular, morphological, and rearing data resulted in the following synonyms: *E. phalangoides* (De Geer, 1778) [= *Erythraeus adrastus* (Southcott, 1961), syn. nov.], *E. cinereus* (Dugès, 1834) [= *Erythraeus jowitae* Haitlinger, 1987, syn. nov.], and *E. regalis* (C.L. Koch, 1837) [= *Erythraeus kuyperi* (Oudemans, 1910), syn. nov., = *Erythraeus gertrudae* Haitlinger, 1987, syn. nov.]. The molecular evidence confirmed the separate identity of three further members of the genus. We provide redescriptions of *E. phalangoides*, *E. cinereus*, and *E. regalis* after modern standards, and neotypes are designated.

Keywords Biology · GMYC · Molecular species delimitation · Morphology · New synonyms · Parasitengona

Introduction

Few organisms look like small exact copies of the sexually mature adult already at birth, that is, some changes other than just isometric growth are commonly associated with the development from juvenile to adult organism. With great change, e.g., through metamorphosis, species-level identification will rely on completely different sets of morphological or anatomical characters for different life history forms. For the huge diversity of arthropods, with vast numbers still to be discovered and described (Erwin 1982; Basset et al. 2012), it is not surprising that most are known exclusively from a single life history form. For arthropods in general, this is commonly the adult but for various groups it can be the larval form (e.g., vertebrate parasitizing Trombiculidae). Connecting a species' life history forms, especially

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when biology changes substantially through development, is of course immensely important for understanding a species function in its ecosystem. As an example, malaria control efforts would look very different today if the life history forms of its vector were not connected. A particular problem arises if separate scientific schools, or scientists during different time periods, name life history forms in isolation of each other's work. This results in a "double classification"—many of the separate adult and larval names must be conspecific since they are based on the same regional fauna but to infer the correct linkage is non-trivial (Łaydanowicz and Małkol 2010). Such is the current situation for erythraeid mites where in the past the taxonomy was largely based on adults but more recently has been based on the parasitic larval forms (Małkol and Wohltmann 2012).

One of the most speciose terrestrial parasitengone genera, *Erythraeus* Latreille, 1806 (Actinotrichida: Parasitengona, Erythraeidae), comprises over 100 (121) nominal species. However, the number of nominal species assigned to the genus today might differ substantially from the true number. A vast majority has been described either from larvae or active postlarval forms (deutonymph and/or adult). Hitherto, 43 larval and 50 postlarval names have been accommodated in the subgenus *Erythraeus*, whereas all 26 species in the subgenus *Zaracarus* Southcott, 1995 are solely known from larvae (Kamran et al. 2011, 2013; Małkol and Wohltmann 2012, 2013; Kamran and Alatawi 2014; Mahmoudi et al. 2014; Haitlinger and Šundić 2015; Šundić et al. 2015a, b). Only two species, namely *Erythraeus nipponicus* Kawashima, 1961 and *Erythraeus styriacus* Turk, 1981, are known from both larvae and adults.

Until recently, the only available method to match larvae and postlarval forms of parasitengone mites was experimental rearing of field-collected ovigerous females or of parasitic larvae (Southcott 1961). Because of sampling effort, captivity stress, and the complexity of factors influencing parasitengone life cycles, such attempts have been rarely performed for Erythraeidae in the past (Małkol and Wohltmann 2012, 2013).

Molecular methods for species identification now offer alternative ways to both study trophic interactions and to correlate life history forms of species (see Kress et al. 2015 for a recent review). Since the seminal paper by Hebert et al. (2003), this is broadly referred to as DNA barcoding, although in its strict sense, this term only applies to when certain standard genetic markers are being used (mitochondrial COI in animals). Larval-adult matching using DNA has been successfully applied to fish (Hubert et al. 2010; Baldwin and Johnson 2014 and references therein), amphibians (Vences et al. 2005), various invertebrate groups (Jousson et al. 1999;

Locke et al. 2011; Okassa et al. 2012; Alcántar-Escalera et al. 2013) including crustaceans (Feller et al. 2013), and a range of insect groups such as beetles (Miller et al. 2005; Caterino and Tishechkin 2006; Ahrens et al. 2007; Levkanicova and Bocak 2009; Lefort et al. 2012), Lepidoptera (Prado et al. 2011), Diptera (Pfenninger et al. 2007; Sutou et al. 2011), caddisflies (Johanson 2007; Zhou et al. 2007; Waringer et al. 2008), mayflies (Gattolliat and Monaghan 2010), stoneflies (Mynott et al. 2011; Avelino-Capistrano et al. 2014), and hemipterans (Zhang et al. 2008; Zhang and Weirauch 2011) to name a few. The main challenge is the unavailability of reference sequences of adults (Pfenninger et al. 2007) or insufficient field sampling of both life instars. Limited amount of DNA that can be obtained from relatively small specimens requires DNA extraction from the entire specimen. However, non-destructive methods of DNA extraction make it possible to retain the exoskeleton for voucher slide preparation (Cruickshank 2002; Dabert et al. 2008). Another challenge can be that the selected molecular marker is not variable enough for species-level identification. This can be due to that the marker itself is conservative with a slow substitution rate or that the species group is relatively young (Hickerson et al. 2006; Bergsten et al. 2012). Mitochondrial genes, like the animal COI barcode, can also portray false species identifications (and false adult-larval linkages) due to hybridization among species and introgression (Whitworth et al. 2007). Despite these challenges, and along with the relatively low cost and time expense, the technique offers an alternative to rearing and is indeed one of the most useful applications of DNA barcoding (Hebert and Gregory 2005; Kress et al. 2015).

In the present study, we test the validity of different *Erythraeus* species described independently as larvae and adult instars. Two separate methods are used to increase the accuracy in adult-larval linkage and to evaluate the applicability of molecular analysis compared with experimental rearing in terrestrial parasitengone mites. As a consequence of the results, we provide re-descriptions for *Erythraeus phalangoides* (De Geer, 1778), *Erythraeus cinereus* (Dugès, 1834), and *Erythraeus regalis* (C.L. Koch, 1837), along with a list of new junior synonyms and neotype designations.

Material and methods

Collecting, mounting, and morphological studies

Larvae and adults of *Erythraeus* spp. were collected at different localities throughout Sweden, Germany and Poland. Data

Table 1 List of collecting places in Sweden (1–12), Germany (13–19), and Poland (20–32)

Locality	Description	Sampling time	Sampling frequency/method	Collector	Species
1. Sorsele	Sandy roadside, Stora Tjulträsk (Trap ID 13–23), 65° 57' 51.8" N, 16° 02' 45.6" E	20 June 2013	Sifting	RH	<i>Erythraeus regalis</i> ^b (2 adults)
2. Vindeln	Edge of bog, Kulbäcksliden (Trap ID 59), 64° 10' 53.9" N, 19° 33' 32.9" E	5–20 August 2004	Malaise trap	SMTP	<i>Curteria episcopalis</i> ^b (1 adult) <i>Erythraeus regalis</i> ^b (1 larva)
3. Munkfors	Near stream Klarälven, pasture with alder forest (Trap ID 13–2), 59° 46' 26.1" N, 13° 27' 52.2" E	5 June 2013	Sifting	JS, MF, RH	<i>Erythraeus regalis</i> ^b (12 adults)
4. Norrtälje	Near lake and wetland, Håjö (Trap ID 11–14), 59° 43' 04.8" N, 18° 53' 17.1" E	22 July 2011	Handpicking	JS	<i>Erythraeus phalangoides</i>
5. Norrtälje	Meadow near forest stream, St. Dvylanda (Trap ID 11–6), 59° 42' 27.98" N, 18° 13' 59.58" E	18 June 2011	Handpicking	JS	<i>Erythraeus phalangoides</i>
6. Håbo	Shore of Lake Mälaren, Skokloster, (Trap ID 13–20), 59° 42' 20.8" N, 17° 31' 59.7" E	15 June 2013	Handpicking	JS, MF	<i>Erythraeus regalis</i> ^b (1 adult)
7. Norrtälje	Maritime deciduous wood, Svartlöga, Matkrok (Trap ID 26), 59° 34' 10.0" N, 19° 03' 10.8" E	15–28 July 2004	Malaise trap	SMTP	<i>Erythraeus regalis</i> ^b (4 larvae)
8. Stockholm	Pathway, Lappkärsberget (Trap ID 9–1), 59° 22' 08.4" N, 18° 03' 54.5" E	31 March 2009	Handpicking	NA	<i>Erythraeus cinereus</i> ^b (3 adults)
9. Stockholm	Near small lake, Lappkäret (Trap ID 13–43), 59° 22' 04.2" N, 18° 04' 09.9" E	23 August 2013	Sifting	JS	<i>Erythraeus cinereus</i> ^b (1 adult)
10. Nacka	Edge of forest, Nacka nature reserve (Trap ID 13–44), 59° 17' 14.6" N, 18° 10' 12.3" E	24 August 2013	Handpicking	JS	<i>Erythraeus cinereus</i> ^b (1 adult)
11. Nybro	Mixed forest near stream, Alsterbro/Alsterån (Trap ID 1008), 56° 56' 11.5" N, 15° 55' 12.6" E	10–15 June 2005	Malaise trap	SMTP	<i>Erythraeus cinereus</i> ^b (2 larvae)
12. Nybro	Meadow in forest edge, Grytsjön nature reserve (Trap ID 1001), 56° 55' 53.1" N, 16° 05' 07.9" E	2–13 August 2005 18 May to 15 June 2006	Malaise trap	SMTP	<i>Erythraeus regalis</i> ^b (12 larvae) <i>Erythraeus cinereus</i> ^b (2 larvae)
13. Bremen	4 sampling sites, ruderal areas on campus or nearby University of Bremen, 53° 06' N, 8° 51' E	April 1990 to September 1995	Irregular intervals, pitfall traps, handpicking	AW	<i>Erythraeus phalangoides</i> <i>Erythraeus regalis</i> ^a <i>Curteria episcopalis</i> <i>Erythraeus phalangoides</i>
14. Harz	7 locations close to relics of peat bog near St. Andreasberg, Harz, 51° 45' N, 10° 30' E	April to October 1997 May 2004 May to July 2005 May to June 2006	Monthly intervals, pitfall traps, handpicking	HBS	<i>Erythraeus cinereus</i> ^a
15. Berlin	4 locations in forested area around Grossbeeren close to Berlin, 52° 22' 07" N, 13° 19' E, lowland riparian forest with annual inundation by nearby creeks during winter time	April to December 1996 January to September 1997 January to September 1998 January to October 1999 February to September 2000 February to May 2001 March to October 2004	Three times a month, sweep net, handpicking	AW	<i>Erythraeus cinereus</i> ^a <i>Erythraeus phalangoides</i> ^a
16. Eifel	5 sampling sites at the northern Eifel near Berg, Zülpich, 50° 38' N, 6° 36' E, calcareous low-nutrient meadows	March 2004 to October 2005	Monthly intervals, pitfall traps, handpicking	HBS	<i>Erythraeus phalangoides</i>
17. Mosel	6 sampling sites at sunny slopes at river Mosel near Koblenz-Gondorf, 50° 19' N, 19° 20' E, ruderal area, in summer Mediterranean climate	March 2004 to October 2005	Monthly intervals, pitfall traps, handpicking	HBS	<i>Erythraeus phalangoides</i>
18. Ahlenmoor	Relicts of bog areas close to Flögelh near Bremerhaven, 53° 41' N, 8° 47' E	1986–2006	Irregular, handpicking, pitfall traps	AW	<i>Erythraeus phalangoides</i>

Table 1 (continued)

Locality	Description	Sampling time	Sampling frequency/method	Collector	Species
19. Charlottenhorst	Garden area at Charlottenhorst near Neubrandenburg, 53° 41' N, 13° 45' E	1997–2001	Irregular, handpicking	AW	<i>Erythraeus phalangoides</i>
20. Gieraków, Stronie Śl.	Meadow, 51° 11' N, 15° 18' E	1 June 1998	Handpicking	JM, BP, AWI	<i>Erythraeus regalis</i> ^a
21. Bieszczady Mts.	Slope on the Solina lake, 49° 19' N, 22° 31' E	10 May 2012	Handpicking	MF, HM, AP	<i>Erythraeus regalis</i> ^{ab} (1 adult)
22. Bieszczady Mts., Krywka	49° 15' N, 22° 44' E	9 May 2012	Handpicking	MF	<i>Erythraeus regalis</i>
23. Bystrzyckie Mts., Niemojów	Dry grass on the small pond, sunny slope with <i>Corylus avellana</i> stand, 50° 09' N, 16° 34' E	19 May 2013 6 June 2014 23–24 May 2015	Handpicking	JM	<i>Erythraeus regalis</i> ^{ab} (1 adult, 4 larvae)
24. Potworów, Bardo Śl.	Meadow, on the slope, grass, and dry leaves, 50° 31' N, 16° 44' E	1 June 1998	Handpicking	JM	<i>Erythraeus cinereus</i> ^a
25. Białowieża Primeval Forest	Flooded valley of the Lesna river, Olszanka–Mysłiszczce nature reserve, 52° 38' N, 23° 40' E	24 September 1998	Handpicking	MW	<i>Erythraeus cinereus</i> ^a
26. Wrocław -Osobowice	Meadow, 51° 09' N, 17° 00' E	9 June 2009	Handpicking	JL	<i>Erythraeus phalangoides</i> ^{ab} (4 larvae)
27. Lubin	Meadow, 51° 23' N, 16° 16' E	1 July 2009	Handpicking	JL, AK	<i>Erythraeus phalangoides</i> ^a
28. Raków, Wrocław	Meadow and reed belt on the fishpond, 51° 10' N, 17° 16' E	4 October 2004	Pitfall traps	AS	<i>Erythraeus cinereus</i>
29. Kamieniec Wrocławski	Allotment garden, 51° 04' N, 17° 11' E	2 July 1992	Handpicking	JM	<i>Erythraeus phalangoides</i>
30. Łosice	Meadow, 51° 13' N, 17° 12' E	14 June 2004	Pitfall traps	AS	<i>Erythraeus phalangoides</i>
31. Boryszyn	Edge of the meadow, 52° 21' N, 15° 27' E	21–28 July 2008	Pitfall traps	JM	<i>Erythraeus phalangoides</i>
32. Wysoka, Boryszyn	On the lake Paklicko Małe, 52° 22' N, 15° 27' E	21–27 July 2008	Pitfall traps	JM	<i>Erythraeus phalangoides</i>

Collectors in the order of the following listing: Rasmus Hovmöller, Swedish Malaise Trap Project, Jeanette Stålstedt, Magdalena Felska, Niklas Apelqvist, Andreas Wohltmann, Hans-Bert Schikora, Joanna Mąkol, Beata M. Pokryszko, Andrzej Wiktor, Hanna Moniuszko, Agnieszka Piróg, Elżbieta Kowalska, Dariusz Lupicki, Marek Wanat, Joanna Łaydanowicz, Aleksandra Klosińska, and Anna Szuba

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^a Populations from which successful experimental rearing resulting in correlation of larvae and active postlarval instars was performed in laboratory

^b Populations from which specimens were selected for molecular analyses (number of specimens)

Table 2 Data on biology of *Erythraeus* spp.

	<i>Erythraeus phalangoides</i>	<i>Erythraeus cinereus</i>	<i>Erythraeus regalus</i>
Field data			
Field occurrence of larvae	April–early June (<i>n</i> = 35)	June–August (<i>n</i> = 234)	Late July–August (<i>n</i> = 50)
Host	Aphididae (<i>n</i> = 5)	Aphididae (<i>n</i> = 122), conspecific larvae (<i>n</i> = 6)	Aphididae, nymphs and adults (<i>n</i> = 24)
Intensity of infestation	1–3	1–(3) ^a	1–(5) ^a
Prevalence	Not recorded	50 % (July)	Not recorded
Site specificity	Not evident	Not evident	Not evident
Field occurrence of protonymphs	Not observed	Not observed	August (<i>n</i> = 1)
Field occurrence of deutonymphs	Late May–July (<i>n</i> = 46)	End of July–end of August (<i>n</i> = 53)	August–March (<i>n</i> = 34)
Field occurrence of tritonymphs	Not observed	August–end of September (<i>n</i> = 16)	May (<i>n</i> = 16)
Field occurrence of adults	June–July (<i>n</i> = 219)	late August–mid June (<i>n</i> = 512)	May–end of June (<i>n</i> = 152)
Laboratory data			
Egg deposition (number of eggs/clusters per clutch)	40–106/1–3 clusters (<i>n</i> = 13)	69–235 eggs/1–4 clusters (<i>n</i> = 31)	78–114 eggs/1–3 clusters (<i>n</i> = 25)
Egg size (µm)	190–220 × 279–275	250–280 × 350–380	250 × 350
Development of eggs into larvae (<i>n</i> = number of eggs)	20 °C/20–45 days, 5 °C/179–317 days, re-exposed to 20 °C/10–15 days (<i>n</i> = 155)	10 °C/150–165 days (<i>n</i> = 35)	20 °C/32–47 days (<i>n</i> = 720)
Maximum survival time of unfed larvae (<i>n</i> = clutch)	20 °C/14 days (<i>n</i> = 52)	20 °C/17 days (<i>n</i> = 10)	20 °C/13 days (<i>n</i> = 60)
Duration of parasitic phase	Not recorded	20 °C/2 days (<i>n</i> = 6)	20 °C/2–5 days (<i>n</i> = 4)
Activity of larva at postparasitic phase	Not recorded	<24 h/optimal conditions (5 days/20 °C–in lack of crevices to hide)	48 h
Onset of protonymph–onset of deutonymph	Not recorded	10 °C/52–67 days (<i>n</i> = 4)	20 °C/17–22 days (<i>n</i> = 15)
Onset of deutonymph–onset of tritonymph	Not recorded	15 °C/22–23 days (<i>n</i> = 5)	20 °C/9–14 days (<i>n</i> = 25)
Onset of tritonymph–onset of adult	20 °C/11 days (<i>n</i> = 1)	20 °C/11–19 days (<i>n</i> = 4)	5 °C/102 days, re-exposed to 20 °C (<i>n</i> = 1)
Prey of active postlarval forms	Formicidae (Hymenoptera)—adults, eggs, and larvae	15 °C/28–41 days (<i>n</i> = 5)	Not recorded
Diapausing instar	Egg	Brachycera (Diptera), Formicidae (Hymenoptera)—larvae and pupae	Brachycera (Diptera), Formicidae (Hymenoptera)—larvae and pupae
		Adult prior to reproduction	Deutonymph

^a Maximum load refers to single cases

on localities, sampling time, method and frequency of sampling, collectors' name, and taxon ID, along with the data on material that underwent successful rearing and material dedicated for molecular studies, including *Curteria episcopalis* (C.L. Koch, 1837) selected as an outgroup, are provided in Table 1. For morphological analyses, larvae and adults selected randomly from each sample period were used. Alcohol-preserved, non-extracted specimens were cleared in potassium hydroxide or in lactic acid prior to mounting. The orcein dye was used in order to stain the exoskeletons of DNA-extracted larvae. The material was mounted on permanent slides in polyvinyl-lactophenol (material from Germany), euparal (material from Sweden), or in Faure's medium (material from Poland). Measurements and drawings were made using Nikon Eclipse 80i, Nikon Eclipse E600, or "B-2" Olympus microscope, equipped with differential interference contrast (DIC) and drawing tube. All measurements are given in micrometers (μm). The total length of legs includes coxae. Terminology, abbreviations applied to morphological structures, and measurement standards follow Wohltmann et al. (2007) and Małol (2010), with the following modifications: N = non-specialized, nude seta (may have minute setules) on palps and legs and B = non-specialized, barbed, or setulated seta on palps and legs. Data on deutonymphs, due to their overall similarity to adults but also due to unrecognized intraspecific variation of metric and meristic data, apply only to the life instar behavior.

As the type material of *E. phalangoides*, *E. cinereus*, and *E. regalis* was unavailable for studies, we referred to original descriptions and complementary data published in an array of papers (De Geer 1778; Dugès 1834; Koch 1837; Oudemans 1910, 1929; Southcott 1961; Haitlinger 1987; Wohltmann et al. 2007), supplemented with reference material from authors' collections. Three further members of the genus (*Erythraeus* sp. 1, *E.* sp. 2, *E.* sp. 3) for which the separate identity was confirmed by means of molecular analyses were considered in the present study in order to strengthen the evidence on the applicability of molecular methods in separation of specific level taxa. The comprehensive characteristics of these species will constitute the subject of a separate study. DNA extractions are stored at the Swedish Museum of Natural History (NHRS), whereas voucher specimens, apart from neotypes (see: type material in systematic part of "Results and discussion"), are at the author's collections. Sequences (*E. phalangoides*, *E. cinereus*, and *E. regalis*) are deposited in GenBank under accession numbers KU892288–KU892385.

Molecular analyses

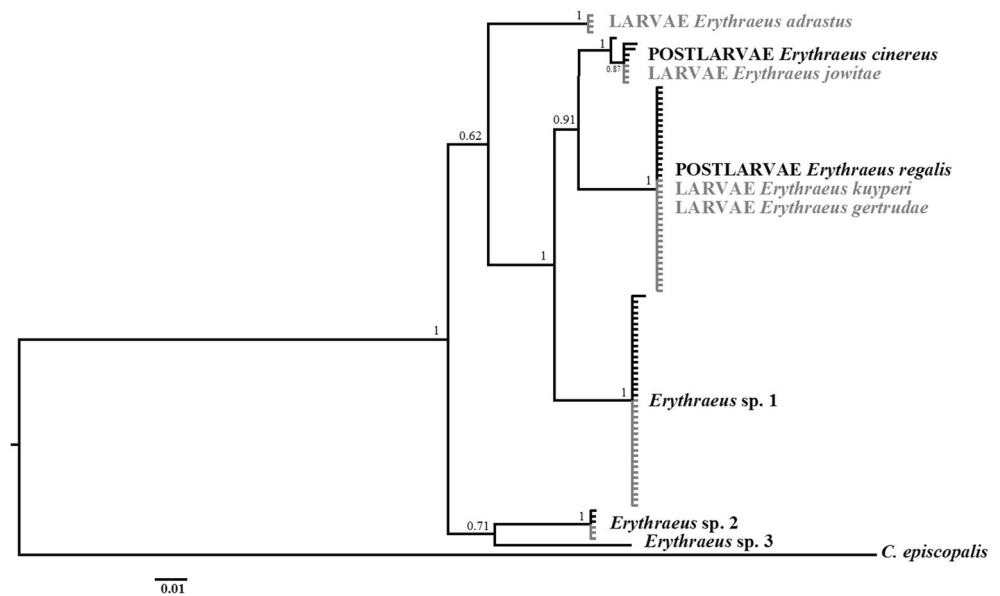
A non-destructive method of DNA isolation was applied to specimens used for molecular analysis (Table 1), which allowed the exoskeletons to be slide mounted

post-extraction (Cruickshank 2002; Dabert et al. 2008). The molecular work was carried out at the Molecular Systematics Laboratory (MSL), Swedish Museum of Natural History, Stockholm. Four legs from one side of mite's body or the whole mite, depending on the size of specimen, were subject to extraction. The extraction method followed the Cell and Tissue DNA Kit protocol of the KingFisher™ Duo (Thermo Scientific). Lysis ($56\text{ }^{\circ}\text{C}$) of larvae included 3–4 h and 15–16 h (overnight) for adults. The D2 region of nuclear 28S rDNA (28S) was amplified using primers D2F forward 5'-AGTCGTGTTGCTTGATAGTGACAG-3' and D2R reverse 5'-TTGGTCCGTGTTTCAAGACGGG-3' (Campbell et al. 1993; Goolsby et al. 2006). PCR cycle and sequencing protocols followed Stålstedt et al. (2013).

The primers amplifying the D2 region of 28S gave a sequence length of 582–587 bp with 116 variable characters, of which 51 were parsimony informative. Sequences with more than 10 % missing data were excluded. The average nucleotide composition were $A=28\%$, $G=27\%$, $T=26\%$, and $C=19\%$.

Raw sequence data were assembled using Sequencher v. 4.10.1 (2008). Primer sequences were removed from the beginning and end of each sequence. The 28S sequences were aligned using FFT-NS-i in MAFFT v.7 (Katoh and Standley 2013) which resulted in an alignment length of 591 bp. In total, 98 samples, including *C. episcopalis* (C.L. Koch, 1837), taxon included as an outgroup, were used to reconstruct a gene tree with Bayesian analysis, using MrBayes v.3.2.4 (Ronquist et al. 2012). The substitution model GTR + Γ was selected with MrModeltest v. 2.3 (Nylander 2004). The prior on branch lengths was set to an exponential distribution with a mean of 0.01 to prevent the exploration of artificially long trees (Brown et al. 2010). Markov chain Monte Carlo (MCMC) ran for 20 million generations, sampled every 500 generations and with the first 25 % of samples discarded as burn-in. The standard deviation of split frequencies between runs was below 0.01 ($=0.0016$). The generalized mixed Yule coalescent model (GMYC) (Pons et al. 2006; Fujisawa and Barraclough 2013) was used as a method of molecular species delimitation on the single-locus gene tree. The GMYC analysis requires an ultrametric tree which was inferred with a clock model in BEAST v.2.1.3 (Bouckaert et al. 2014). Duplicate haplotypes were removed from the matrix using DNACollapser (Villesen 2007) since identical sequences can cause problems with the GMYC model (Fujisawa and Barraclough 2013). The outgroup was excluded as the clock model roots the tree. The analysis contained 45 haplotypes and the settings were GTR + Γ , strict clock, coalescent

Fig. 1 Majority-rule consensus from the non-clock Bayesian phylogenetic analysis of 28S from *Erythraeus* larvae and postlarval instars sampled in the field. Numbers above branches denote posterior probability values. The clusters of *Erythraeus cinereus*, *E. regalis*, *E. sp. 1*, and *E. sp. 2* have both larvae (gray) and adults (black) included. *Curteria episcopalis* was used as outgroup to root the tree



constant population tree prior, and 20 million generations (MCMC) with 25 % burn-in. The GMYC analysis was performed in R version 3.1.3 with the “splits” package (Ezard et al. 2014; R Core Team 2015). Nucleotide composition statistics, genetic intra- and interspecific distances (Kimura 1980), and parsimony informative characters were obtained using MEGA v.6.06 (Tamura et al. 2013).

Experimental rearing

Experimental rearing aimed at obtaining larvae from field-collected females and recognition of the diapausing instar of particular species. Live specimens were kept in laboratory in polystyrene boxes (25 × 25 × 20 mm)

(material from Germany) or glass vials (24 × 34 mm) covered with semi-transparent lid (material from Poland) and filled to ca 2/3 with charcoaled Plaster-of-Paris. The material was kept at controlled temperature (20 °C, ±1 °C tolerance) and light (12 h dark, 12 h light) in environmental chambers. In case of successful development of eggs into larvae, the recorded rearing success was >90 %. When development stopped at a certain instar, specimens were exposed to 5 °C for about 90 days and subsequently re-exposed to 20 °C in order to imitate winter conditions and to break diapause. Saturated air humidity and humidity of the substratum (indicated by changes in color of charcoaled Plaster-of-Paris) were attained by the addition of water to the vial. Test on the influence of humidity followed

Fig. 2 Ultrametric clock tree from Beast showing the maximum likelihood solution of the generalized mixed Yule coalescent model (GMYC). Red lines (thinner lines on the right side of the graph) indicate within-species branches, and black lines (thicker lines on the left side of the graph) represent between-species branches as inferred by the GMYC model

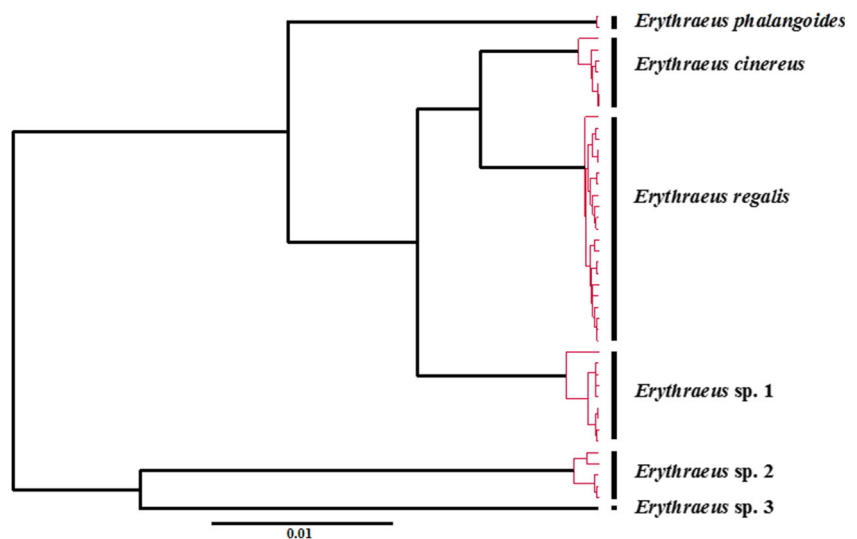
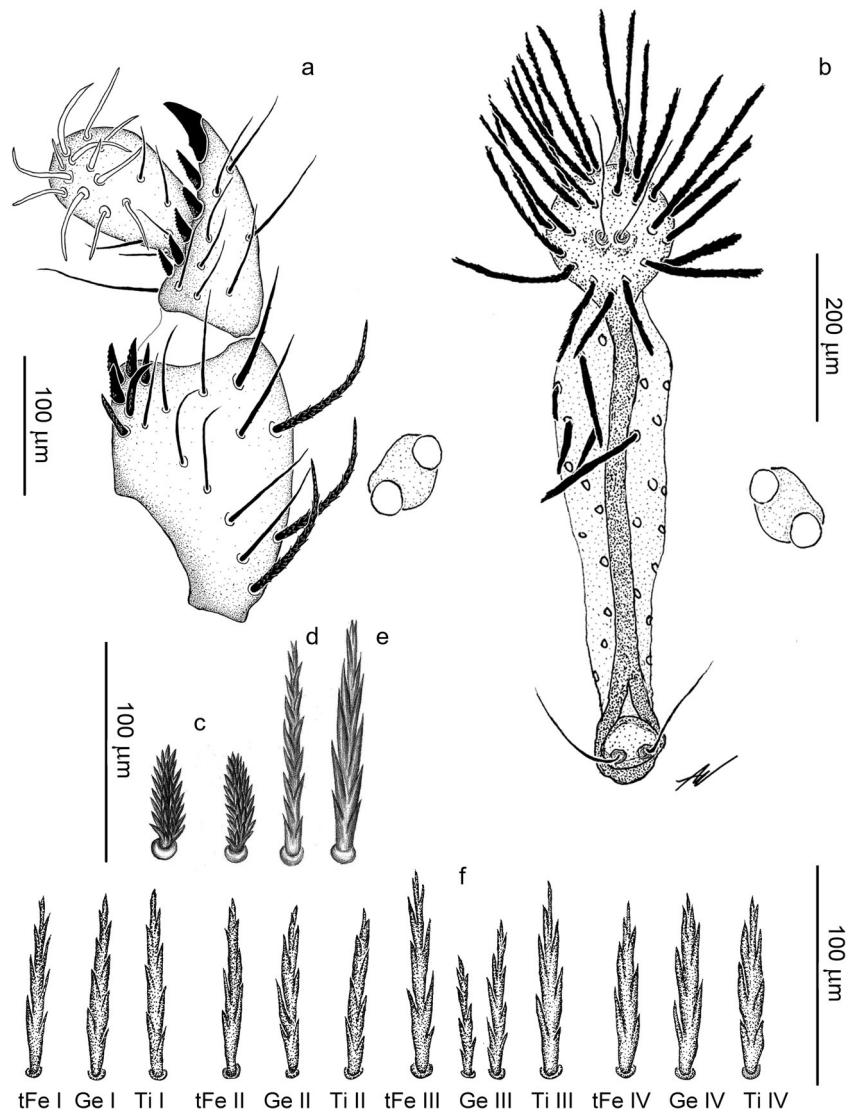


Fig. 3 *Erythraeus phalangoides*, adult. **a** Palp, medial view. **b** Crista metopica and eyes. **c** Dorsal opisthosomal setae. **d** Serratala on genu I. **e** Serratala on genu IV. **f** Diversity of serratalae on telofemora, genua, and tibiae of legs I–IV



procedures described in Wohltmann (1998). Aphididae were offered as hosts to all three *Erythraeus* species, while larvae of *E. cinereus* were also given the immature forms of Formicidae as well as deutonymphs and adults of Miridae. Active postlarval forms of *E. cinereus* and *E. regalis* were offered larvae and pupae of Brachycera (Diptera) and Formicidae (Hymenoptera) as prey.

Results and discussion

Field data

Erythraeus spp. was sampled at various locations in Sweden, Poland, and Germany (Table 1). Seasonal abundance of instars (Table 2) did not differ within a particular species

sampled at various localities. Postlarval mobile instars were active on soil surface and in the litter layer. Larvae were collected either as free-living, during host search, or attached to aphid host during parasitic phase (Table 2). Only few of the immobile instars were collected in crevices. Edaphic occurrence as reported for other Parasitengona (Wohltmann 2000 and references therein) was not observed in any of the *Erythraeus* spp. In case of syntopic occurrence of *Erythraeus* sp. (10 % of studied cases—see Table 1), species usually differed in the annual abundance of instars. Data on habitat preferences and details concerning life history patterns of *Erythraeus* spp. are provided in systematic part of “Results and discussion” and in Tables 1 and 2. Patterns concerning phenology and biology of *Erythraeus* spp., observed during present study, confirm earlier reports on European members of the genus (Beron 1982; Wohltmann 2000, 2001) and fit also general data provided by Southcott (1961, 1988).

Table 3 Metric data on adults of *Erythraeus* spp.

Character	<i>Erythraeus phalangoides</i>					<i>Erythraeus cinereus</i>					<i>Erythraeus regalis</i>				
	Total (n = 14) min-max	Germany (n = 5) min-max	Sweden (n = 3) min-max	Poland (n = 6) min-max	Total (n = 16) min-max	Germany (n = 7) min-max	Sweden (n = 5) min-max	Poland (n = 4) min-max	Total (n = 18) min-max	Germany (n = 2) min-max	Sweden (n = 10) min-max	Poland (n = 7) min-max			
Gnathosoma															
PaTr	100-130	100-130	100-110	105-127	95-125	95-110	100-125	100-107	93-125	95-110	94-115	93-125			
PaFe	255-380	320-380	285-330	255-371	320-390	320-375	345-370	363-390	280-397	280-310	295-375	326-397			
PaGe	175-230	200-230	175-205	185-225	194-224	200-220	200-220	194-224	170-230	170-195	172-230	193-230			
PaTi	125-182	140-180	125-165	145-182	130-179	130-165	151-165	154-179	125-180	125-155	139-180	147-175			
Odonus	30-50	30-40	45-50	36-50	35-69	35-60	42-51	52-69	24-49	24-34	31-49	31-49			
PaTa	117-150	125-150	130-150	117-145	125-158	125-155	140-158	138-154	100-150	100-125	105-150	105-141			
Idiosoma															
CML	628-874	685-825	650-760	628-874	590-800	680-800	590-705	716-792	505-822	505-695	510-720	640-822			
ASens	133-200	135-200	145-150	133-158	150-225	150-225	185-204	181-196	135-192	135-175	153-192	163-186			
PSens	150-195	150-195	155-165	168-186	187-235	200-225	187-229	195-235	161-211	175-210	161-206	182-211			
SBa	19-35	25-35	20-25	19-35	20-36	25-27	20-36	21-32	20-37	22-25	23-37	20-30			
SBp	18-33	25-30	20-33	18-32	25-36	25-35	30-36	28-33	25-39	25-27	31-38	25-39			
ISD	495-700	580-700	495-685	560-675	509-605	515-605	515-595	509-604	420-643	420-490	420-630	475-643			
pDS	22-70	30-70	22-50	25-56	42-215	42-190	51-119	79-215	50-176	68-130	50-155	55-176			
Legs															
Cx I	475-700	600-700	475-600	496-655	460-554	460-500	475-550	492-554	400-610	400-475	430-610	490-573			
Tr I	220-282	240-250	220-230	226-282	170-300	170-250	240-300	227-259	175-335	175-205	240-335	206-300			
bFe I	336-500	365-500	360-380	336-436	300-440	300-420	360-440	334-440	319-460	350-385	350-460	319-450			
tFe I	550-713	660-710	550-600	614-713	543-680	590-610	600-680	543-611	490-730	525-575	540-730	490-654			
Ge I	666-820	750-820	620-770	666-818	727-840	750-800	780-840	727-764	625-850	625-650	650-850	648-780			
Ti I	650-870	750-850	650-770	706-870	667-820	700-800	740-820	667-759	625-880	625-665	660-880	675-820			
Ta I (L)	480-610	480-610	490-570	527-604	450-540	450-520	485-540	490-523	400-590	425-485	400-590	458-550			
Ta I (H)	170-220	200-210	170-220	182-219	135-210	135-210	170-200	192-208	150-210	160-175	150-210	171-206			
Leg I	3410-4440	4070-4440	3410-3900	3582-4286	3580-4170	3580-3705	3770-4170	3595-3908	3125-4370	3125-3435	3280-4370	3404-4080			
Cx II	497-646	525-590	500-570	497-646	450-565	500-525	490-530	521-565	400-610	400-480	420-610	500-558			
Tr II	200-300	210-300	200-260	252-296	150-310	150-220	250-310	218-256	204-310	230-285	230-310	204-300			
bFe II	250-331	250-315	270-290	281-331	200-320	200-300	220-320	276-311	200-350	200-265	230-350	260-340			
tFe II	340-550	480-550	340-410	441-508	380-475	380-475	410-450	405-440	350-480	350-410	350-480	368-455			
Ge II	380-550	500-550	380-470	430-548	450-520	450-510	500-520	484-504	375-570	375-485	410-570	420-520			
Ti II	480-760	610-760	480-620	625-741	570-660	570-625	600-660	577-615	525-750	525-620	550-750	560-690			
Ta II (L)	310-485	400-450	310-410	395-485	300-390	300-390	330-385	338-380	300-420	300-335	300-420	330-400			
Ta II (H)	100-152	110-120	100-150	136-152	90-148	90-120	120-140	139-148	100-150	130-150	100-140	120-137			
Leg II	2490-3486	3240-3360	2490-3030	2934-3486	2720-3130	2720-2880	2900-3130	2865-3026	2370-3450	2370-2880	2490-3450	2693-3250			
Cx III	450-700	590-700	450-660	475-656	420-520	420-430	440-520	469-512	430-588	500-525	430-560	490-588			
Tr III	210-300	210-300	220-260	214-274	190-310	190-200	260-310	254-271	200-340	200-280	250-340	238-300			
bFe III	300-400	300-400	300-340	322-368	250-360	250-350	310-360	310-347	250-390	250-310	270-390	282-370			
tFe III	420-621	570-610	420-510	568-621	450-540	450-500	490-540	459-505	400-610	400-550	420-610	460-590			
Ge III	460-750	650-750	460-560	578-670	556-696	590-650	610-640	556-696	490-720	490-625	490-720	510-680			
Ti III	680-1012	960-1000	680-820	827-1012	800-930	800-875	880-930	830-906	750-1080	750-880	810-1060	824-1080			
Ta III (L)	350-543	475-500	350-480	454-543	275-420	275-400	360-420	365-419	290-460	325-420	290-460	380-452			
Ta III (H)	100-210	110-210	100-140	133-147	100-133	100-125	110-130	111-133	75-136	75-105	85-130	100-136			
Leg III	2880-4260	3860-4260	2880-3630	3481-4109	3260-3720	3260-3320	3400-3720	3350-3526	2925-4080	2925-3590	2960-4080	3307-3960			
Cx IV	530-800	720-800	530-770	618-796	500-675	500	580-675	552-629	400-690	400-550	500-690	569-680			
Tr IV	230-390	300-390	230-340	247-331	220-350	220-300	310-350	272-323	220-370	220-310	270-370	270-353			
bFe IV	569-708	580-680	560-620	569-708	450-550	450-510	470-550	450-504	500-730	500-610	540-730	537-669			
tFe IV	815-1108	925-1100	815-900	937-1108	690-850	690-790	750-850	741-826	770-1140	820-875	770-1140	820-1060			
Ge IV	900-1180	950-1180	900-930	916-1179	950-1250	950-1250	1020-1110	969-1033	825-1240	825-1050	870-1240	945-1200			
Ti IV	1430-2087	1750-1950	1430-1630	1770-2087	1400-1650	1400-1520	1460-1650	1464-1541	1500-2200	1500-1850	1500-2120	1615-2200			
Ta IV (L)	600-780	700-780	600-690	637-767	400-530	400-520	470-530	502-521	410-650	475-550	410-650	511-590			
Ta IV (H)	125-176	125-175	130-150	155-176	100-145	100-110	100-130	108-145	100-155	125-130	100-150	120-155			

Table 3 (continued)

Character	<i>Erythraeus phalangoides</i>				<i>Erythraeus cinereus</i>				<i>Erythraeus regalis</i>			
	Total (<i>n</i> = 14) min-max	Germany (<i>n</i> = 5) min-max	Sweden (<i>n</i> = 3) min-max	Poland (<i>n</i> = 6) min-max	Total (<i>n</i> = 16) min-max	Germany (<i>n</i> = 7) min-max	Sweden (<i>n</i> = 5) min-max	Poland (<i>n</i> = 4) min-max	Total (<i>n</i> = 18) min-max	Germany (<i>n</i> = 2) min-max	Sweden (<i>n</i> = 10) min-max	Poland (<i>n</i> = 7) min-max
Leg IV	5185–6835	6070–6835	5185–5850	5744–6746	5070–5665	5070–5160	5120–5665	5105–5199	4670–6840	4670–5795	4860–6840	5309–6640
IP	14,355–18,490	17,140–18,895	14,355–16,410	15,740–18,490	14,630–16,685	14,630–15,065	15,240–16,685	15,078–15,578	13,090–18,670	13,090–15,700	13,590–18,670	14,713–17,930

Molecular data

The gene tree from the Bayesian analysis grouped the sequences into five distinct clusters and one singleton (Fig. 1). Four clusters contained sequences from both larvae and adults ($n=87$) (Fig. 1). All clusters had strong support (>0.95 posterior probability). The GMYC model delimited six to nine entities within a 2 log likelihood unit confidence interval. The maximum likelihood solution was six entities and had a significantly better fit than the null model of a single species ($\log L=400.5$ versus 383.4, $df=2$, p value=0.0008). The six separate units matched the five distinct clusters and the one singleton observed in the non-clock analysis (Figs. 1 and 2). Larvae identified as *Erythraeus kuyperi* and *Erythraeus gertrudae* was inferred to be conspecific with adults identified as *E. regalis*. Larvae of *Erythraeus jowitae* were inferred to be conspecific with adults identified as *E. cinereus*. Species-level taxonomic identification of specimens used for the molecular analyses was unambiguous for *E. cinereus* and *E. regalis*, whereas *E. sp. 1* and *sp. 2* require further taxonomic study. *E. phalangoides* (studied with larvae identified as *Erythraeus adrastus*) and *E. sp. 3* were represented by one instar only (larvae and nymph, respectively); thus, no DNA-based life history form linkage could be inferred for these. The genetic variation within the GMYC-delimited species was very low (0–0.5 %). The genetic divergence between clusters was much larger,

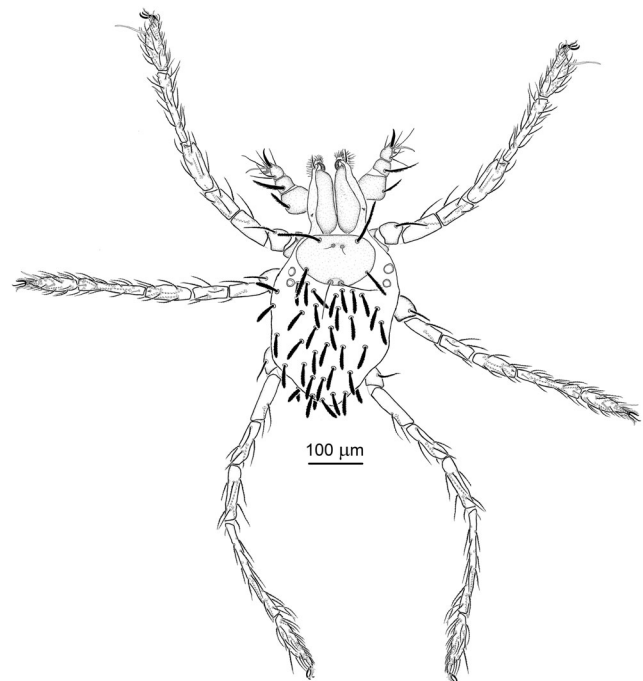
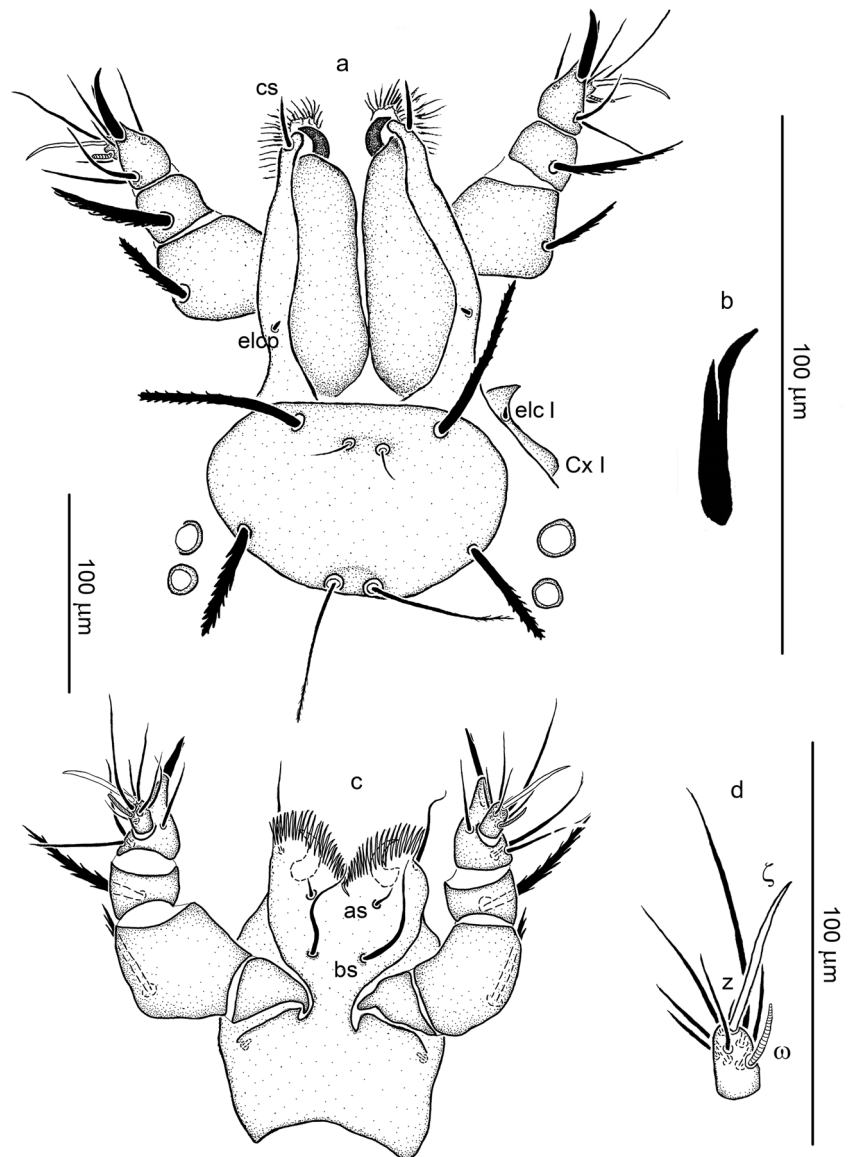


Fig. 4 *Erythraeus phalangoides*, larva. Habitus

Fig. 5 *Erythraeus phalangoides*, larva. **a** Gnathosoma (and scutum), dorsal view. **b** Odontus. **c** Gnathosoma, ventral view. **d** Palp tarsus



between 2.8 and 5.5 %. *E. cinereus* was separated from *E. regalis*, *E. phalangoides* and from *Erythraeus* sp. 1 with 2.8 %. A genetic distance of 4.5 % separated *E. cinereus* from *E. sp. 2* and 5.5 %—from *E. sp. 3*. *E. regalis* and *E. sp. 1* were separated by 3.7 %. The outgroup *C. episcopalis* was separated from the ingroup with 20 %.

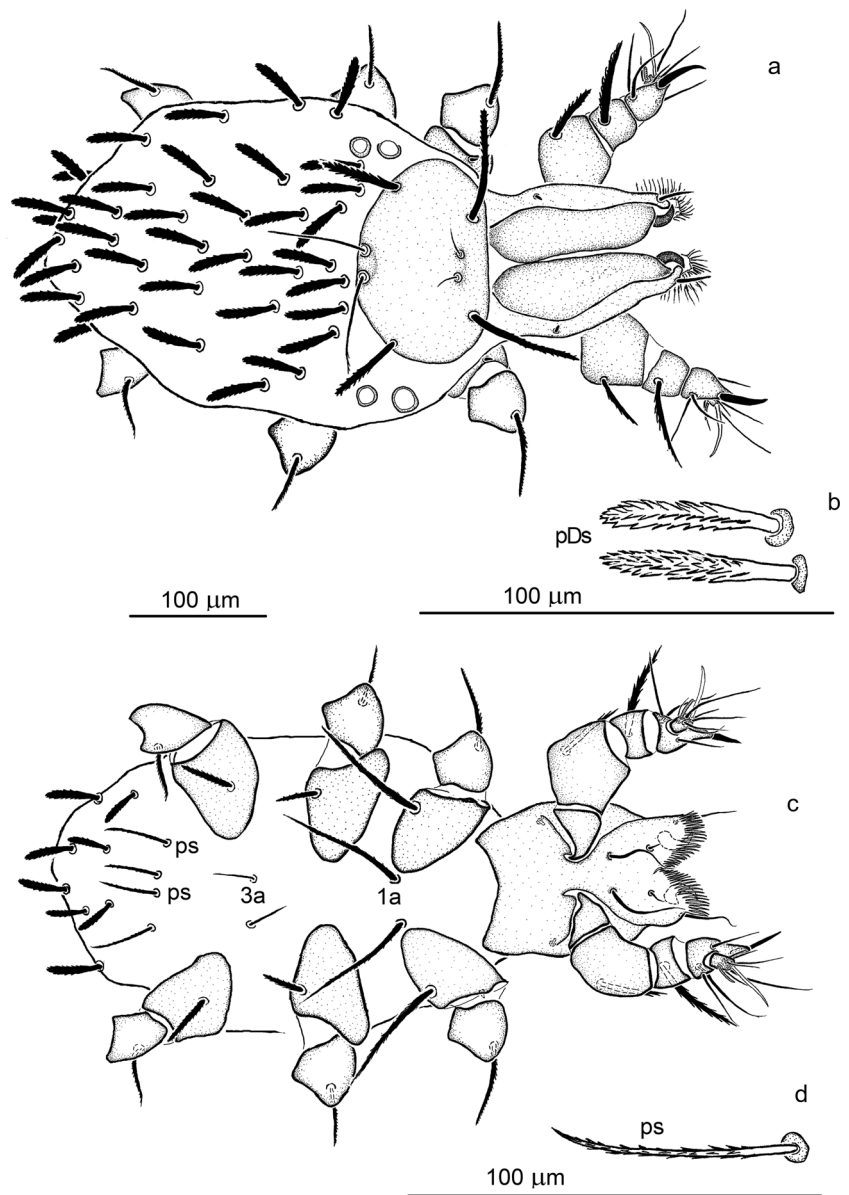
The GMYC model of molecular species delimitation analytically confirmed the visual intuition of clusters from the Bayesian analysis as separate species (Fig. 2). The genetic distances between larvae and adults here inferred to be conspecific were also very low (0–0.5 %). In contrast, the lowest interspecific distance was 2.8 %. In comparison to the mitochondrial barcoding gene COI, nuclear D2 region of 28S is a more conservative gene region and is expected to show less variation (Stålstedt et al. 2013). The inter-cluster distances are

therefore too high to reflect within-species variation. Levels of D2 28S differentiation between species were lower than observed in aquatic Parasitengona of the genera *Hygrobatas* C.L. Koch, 1837, *Piona* C.L. Koch, 1842, and *Unionicola* Haldeman, 1842 (Martin et al. 2010; Stålstedt et al. 2013) but similar to those recorded for the majority of currently recognized congeneric species, including other actinotrichid mites (Skoracka and Dabert 2010; Skoracka et al. 2013). The intraspecific variation was within the range found for other Parasitengona species (Stålstedt et al. 2013).

Rearing data

Successful laboratory rearing was carried out for each of the three *Erythraeus* spp. sampled in the field (Tables 1 and 2). Eggs were ovoid and orange just after deposition but turned

Fig. 6 *Erythraeus phalangoides*, larva. **a** Gnathosoma and idiosoma, dorsal view. **b** Dorsal opisthosomal setae. **c** Gnathosoma and idiosoma, ventral view. **d** Seta *ps*



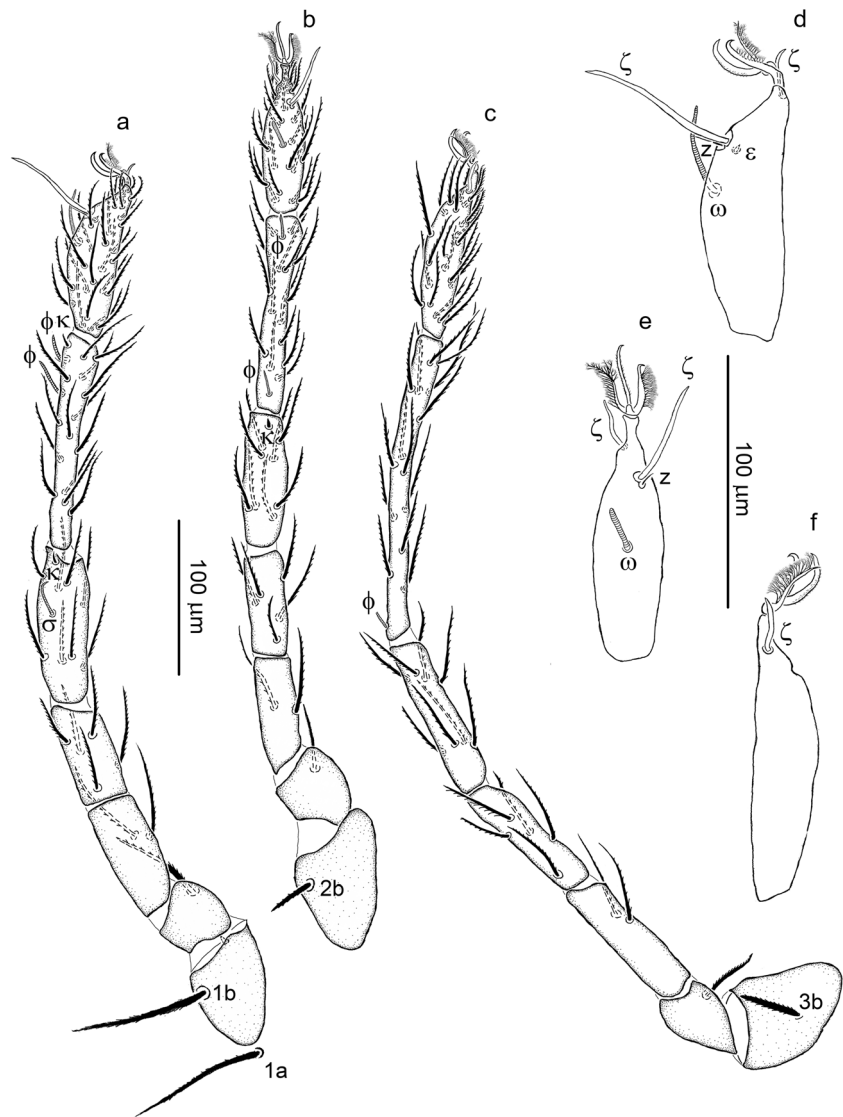
black within 24 h. Larvae hatched about a month later in *E. cinereus* and *E. regalis*, while eggs of *E. phalangoides* underwent obligatory diapause and developed into prelarvae and subsequently into larvae only when kept at 5 °C for the period amounting to about 6–10 months, followed by re-exposure to 20 °C. After hatching, larvae displayed positive phototactic response. Unfed larvae survived without hosts for a few days only (Table 2). When kept together with aphids, larvae readily started parasitism. Fully engorged larvae moved comparably fast but did not display positive phototactic response. Instead, they tried to hide in crevices, where they became motionless and entered the calyptostatic protonymph. Deutonymphs and adults were fed with larvae and pupae of brachyceran flies and ants. In *E. regalis*, the deutonymphs

displayed obligatory diapause and continued development into subsequent instars only after chilling at 5 °C for about 100 days. For *E. cinereus*, the obligatory diapause was recorded in adults, before the onset of reproduction. Additional laboratory data on *E. phalangoides*, *E. cinereus*, and *E. regalis* are provided under respective species description and in Table 2. Data on biology and life history patterns confirm earlier reports by Wendt (1996) and Wohltmann (2000, 2001), applying to *Erythraeus* spp.

Systematic part

Two-way linkage of larvae and postlarval forms—DNA analysis of field-collected specimens and/or laboratory rearing of

Fig. 7 *Erythraeus phalangoides*, larva. **a** Leg I. **b** Leg II. **c** Leg III. **d** Tarsus I. **e** Tarsus II. **f** Tarsus III (**d–f**, only specialized setae shown)



larvae from ovigerous females—resulted in the following synonyms: *E. phalangoides* (De Geer, 1778) [= *E. adrastus* (Southcott, 1961), syn. nov.], *E. cinereus* (Dugès, 1834) [= *E. jowitzae* Haitlinger, 1987, syn. nov.], and *E. regalis* (C.L. Koch, 1837) [= *E. kuyperi* (Oudemans, 1910), syn. nov., = *E. gertrudae* Haitlinger, 1987, syn. nov.]. The following redescriptions include variation of morphological and life history traits between different populations (Sweden, Poland, and Germany) of the particular species. In order to maintain the stability of nomenclature, we are of the opinion that the names of species described in the eighteenth and nineteenth century with an insufficient set of characters in the original descriptions should nevertheless be retained due to their extensive usage and, since they do not contradict the present knowledge of the taxa in question, be supported by much more extended characteristics.

Erythraeidae Robineau-Desvoidy, 1828

Erythraeinae Robineau-Desvoidy, 1828

Other subfamilies: Abrolophinae Witte, 1995, Balaustiinae Grandjean, 1947, Callidosomatinae Southcott, 1957, Leptinae Billberg, 1820, Myrmicotrombiinae Southcott, 1957, Phanolophinae Southcott, 1946a

Type genus: *Erythraeus* Latreille, 1806

Erythraeus Latreille, 1806

For synonymy, see Southcott (1961) and Beron (2008).

Diagnosis

Adult (after Southcott (1961), with modifications, after Wohltmann et al. (2007)): Crista metopica set on indistinct scutum, with two sensillary areas set apart, close to extreme parts of crista; each sensillary area with a pair of trichobothria; two pairs of eyes, each pair inserted in ocular plate; genital plates located at the level of coxae III–IV, distinctly larger than anal sclerites; on medial

Table 4 Metric data on larvae of *Erythraeus* spp.

Character	<i>Erythraeus phalangoides</i>				<i>Erythraeus cinereus</i>				<i>Erythraeus regalis</i>			
	Total (n = 26) min-max	Germany (n = 10) min-max	Poland (n = 16) min-max	Total (n = 22) min-max	Germany (n = 8) min-max	Sweden (n = 4) min-max	Poland (n = 10) min-max	Total (n = 10) min-max	Germany (n = 2) min-max	Sweden (n = 11) min-max	Poland (n = 15) min-max	
Gnathosoma												
PaTr	14-35	14-35	21-31	22-40	22-40	31-34	26-32	24-31	30	24-29	24-31	
PaFe	50-81	50-79	69-81	84-103	84-100	95-103	85-98	70-97	70-90	81-97	73-91	
PaGe	22-41	22-36	29-41	34-50	34-50	46-48	45-48	35-47	38-40	36-47	35-44	
PaTi	30-49	30-47	30-49	39-59	46-58	39-57	54-59	31-56	42-44	34-48	31-56	
Odonatus	32-44	32-40	32-44	42-56	42-56	43-48	47-53	38-52	40-41	42-46	38-52	
PaTa	13-20	14-20	13-20	16-30	16-30	22-26	23-29	19-26	20	20-26	19-23	
Idiosoma												
Scutum_L	85-110	85-110	99-116	119-157	119-138	140-157	125-146	106-145	128-130	114-145	106-126	
Scutum_W	124-173	124-160	152-173	180-213	180-195	192-202	193-213	161-210	184-190	178-210	161-181	
ASens	14-32	14-21	17-32	60-90	60-90	65-78	65-77	50-76	60-64	61-76	50-72	
PSens	60-81	60-75	62-81	84-118	84-110	105-118	93-100	82-110	82-96	87-110	86-98	
SBa	14-24	14-20	14-24	15-23	15-23	17-19	16-23	12-21	16-18	14-21	12-18	
SFBp	17-27	17-22	19-27	16-26	16-26	22-26	17-23	15-25	18-20	15-20	15-20	
AL	65-95	65-90	75-95	115-146	115-142	131-139	135-146	103-134	120-124	113-134	103-121	
PL	50-74	50-65	58-74	100-130	100-130	121-129	102-127	91-122	110	105-122	91-107	
AW	64-89	64-86	70-89	50-71	50-60	60-63	57-71	44-73	50-60	47-73	44-56	
PW	100-127	100-118	115-127	110-132	110-130	117-127	120-132	98-139	110-130	115-139	98-119	
ISD	63-75	68-74	63-75	74-93	74-85	78-93	81-89	68-88	77-82	73-88	68-80	
pDS	25-60	25-60	34-56	59-143	60-140	59-139	69-143	58-127	80-120	58-127	63-119	
Legs												
Cx I	53-80	54-70	53-80	70-102	70-90	94-101	85-102	70-98	80	73-98	70-86	
Tr I	42-58	42-58	45-55	70-85	76-85	74-80	70-82	59-75	60	60-75	59-69	
bFe I	65-84	65-84	71-84	140-180	140-180	155-161	141-162	110-140	140	123-136	110-135	
iFe I	54-72	54-72	58-70	138-162	140-162	147-155	138-160	99-130	116	110-130	99-116	
Ce I	82-110	82-110	95-110	205-250	205-240	225-250	214-232	153-195	180	179-195	153-181	
Ti I	98-141	98-138	119-141	282-340	282-330	318-340	318-326	208-275	274	243-275	208-258	
Ta I (L)	88-115	88-110	99-115	166-210	166-208	200-210	181-193	129-183	164	160-183	129-172	
Ta I (H)	27-37	27-37	29-34	25-32	28-32	25-30	25-29	23-30	25	23-29	23-29	
Leg I	489-636	489-616	579-636	1129-1296	1129-1278	1227-1296	1161-1231	834-1071	1014	976-1071	834-985	
Cx II	65-91	65-73	70-91	100-128	100-117	105-120	109-128	92-116	100	98-116	92-106	
Tr II	47-60	48-59	47-60	66-90	74-90	75-80	66-84	57-76	80	57-76	57-76	
bFe II	56-78	62-78	56-73	129-160	140-160	136-148	129-140	92-117	116	102-117	92-110	
iFe II	54-75	54-70	56-75	129-145	130-144	131-145	129-145	90-115	110	97-115	90-104	
Ce II	70-96	70-90	78-96	175-205	175-197	180-205	179-196	125-155	148	139-155	125-149	
Ti II	97-131	97-126	106-131	285-340	285-334	309-340	304-326	198-255	248	220-255	198-238	
Ta II (L)	80-107	80-100	87-107	160-192	160-192	180-190	163-183	115-168	152	134-168	115-161	
Ta II (H)	22-35	22-35	27-34	25-30	28-30	25-26	23-30	23-30	30	23-30	24-27	
Leg II	478-614	478-569	545-614	1085-1218	1085-1206	1140-1218	1103-1182	806-969	954	863-969	806-959	
Cx III	61-72	61-72	76-89	96-132	96-115	115-125	109-132	80-115	100	95-115	80-112	
Tr III	48-62	50-62	48-59	72-90	72-90	75-80	75-86	57-77	60	62-77	57-76	
bFe III	74-95	74-95	74-93	158-195	174-195	165-173	158-181	110-150	150	126-141	110-136	
iFe III	71-98	71-98	71-95	159-190	170-190	168-180	159-184	112-145	140	125-145	112-136	
Ce III	80-119	80-114	98-119	218-255	220-240	232-255	218-250	151-190	186	165-190	151-186	
Ti III	145-200	145-200	156-200	416-485	416-482	455-485	441-477	310-385	370	328-385	310-370	
Ta III (L)	90-124	90-124	103-124	177-225	177-225	200-220	183-203	127-181	170	153-181	127-177	
Ta III (H)	22-29	22-29	22-27	20-30	24-30	20-25	20-24	18-25	20	18-24	18-24	
Leg III	581-745	581-728	673-745	1367-1517	1382-1517	1435-1513	1367-1488	1014-1223	1176	1059-1223	1014-1176	
IP	1548-1987	1548-1900	1797-1987	3630-4027	3636-4001	3818-4027	3630-3892	2825-3263	3144	2898-3263	2825-3121	

Table 5 Meristic data on adults and larvae of *Erythraeus* spp.

Adults	<i>Erythraeus phalangoides</i>			<i>Erythraeus cinereus</i>			<i>Erythraeus regalis</i>		
	Germany (n = 5)	Sweden (n = 3)	Poland (n = 9)	Germany (n = 7)	Sweden (n = 5)	Poland (n = 4)	Germany (n = 2)	Sweden (n = 10)	Poland (n = 6)
Idiosoma									
AL setae	18–28		15–20	20–30	7–15	10–14	10–12	8–10	9–12
Palps									
Conalae on PaGe	4–6	2–3	2–3	2–5	0	0	0	1–2	0–3
	0–2	0–1	0–1	1–3	0–2	0–4	0–1	0–2	0–2
Semiconalae on PaGe									
Conalae on PaTi	5–6	3–4	3–4	4–6	5	4–6	4–5	5	3–5
Semiconalae on PaTi	0–1	0–1	0–1	0–1	0–1	0	0–1	0–1	0–2
Larvae									
	Germany (n = 10)	Sweden (n = 0)	Sweden (n = 10)	Poland (n = 5)	Germany (n = 2)	Sweden (n = 11)	Poland (n = 8)		
Idiosoma									
NDV	48–52	–	42–48	46–48	54–58	46–50	46–48	50–52	42–50
fd	34–36	–	34–36	36–41	40–44	34–38	36–41	40–42	32–40
Palps									
PaTr	0	–	–	0	0	0	0	0	0
PaFe	1 B	–	–	1 B	1 B	1 B	1 B	1 B	1 B
PaGe	1 B	–	–	1 B	1 B	1 B	1 B	1 B	1 B
PaTi	3 B	–	–	3 B	3 B	3 B	3 B	3 B	3 B
PaTa	6 N, 1 w, 1 ζ, 1 z	–	–	6 N, 1 w, 1 ζ, 1 z	6 N, 1 w, 1 ζ, 1 z	6 N, 1 w, 1 ζ, 1 z	6 N, 1 w, 1 ζ, 1 z	6–7 N, 1 w, 1 ζ, 1 z	6 N, 1 w, 1 ζ, 1 z
Legs									
Cx I	1 N, 1 <i>elc</i>	–	–	1 N, 1 <i>elc</i>	1 N, 1 <i>elc</i>	1 N, 1 <i>elc</i>	1 N, 1 <i>elc</i>	1 N, 1 <i>elc</i>	1 N, 1 <i>elc</i>
Tr I	1 N	–	–	1 N	1 N	1 N	1 N	1 N	1 N
bFe I	2 N	–	–	2 N	3 N	3 N	3(4) N	3 N	3 N
tFe I	5 N	–	–	5 N	5 N	5 N	5 N	5 N	5 N
Ge I	8(7) N, 1 σ, 1 κ	–	–	8 N, 1 σ, 1 κ	8 N, 1 σ, 1 κ	8 N, 1 σ, 1 κ	8 N, 1 σ, 1 κ	8 N, 1 σ, 1 κ	8 N, 1 σ, 1 κ
Ti I	14(13) N, 2(3) φ, 1 <i>da</i> , 1 κ	–	–	14 N, 2 φ, 1 <i>da</i> , 1 κ	14 N, 2 φ, 1 <i>da</i> , 1 κ	14 N, 2 φ, 1 <i>da</i> , 1 κ	14 N, 2 φ, 1 <i>da</i> , 1 κ	14 N, 2 φ, 1 <i>da</i> , 1 κ	14 N, 2 φ, 1 <i>da</i> , 1 κ
Ta I	20–26 N, 1 w, 1 ε, 2 ζ, 1 z	–	–	22–25 N, 1 w, 1 ε, 2 ζ, 1 z	25–26 N, 1 w, 1 ε, 2 ζ, 1 z	22–25 N, 1 w, 1 ε, 2 ζ, 1 z	24 N, 1 w, 1 ε, 2 ζ, 1 z	26 N, 1 w, 1 ε, 2 ζ, 1 z	22–26 N, 1 w, 1 ε, 2 ζ, 1 z
Cx II	1 N	–	–	1 N	1 N	1 N	1 N	1 N	1 N
Tr II	1 N	–	–	1 N	1 N	1 N	1 N	1 N	1 N
bFe II	2 N	–	–	2 N	3 N	3 N	3 N	3 N	3 N
tFe II	5 N	–	–	5 N	5(4) N	5 N	5(6) N	5 N	5 N
Ge II	8(7) N, 1 κ	–	–	8 N, 1 κ	8 N, 1 κ	8 N, 1 κ	8 N, 1 κ	8 N, 1 κ	8 N, 1 κ
Ti II	13–16 N, 2 φ	–	–	13–15 N, 2 φ	15(14) N, 2 φ	15 N, 2 φ	15 N, 2 φ	15 N, 2 φ	15 N, 2 φ
Ta II	19–22 N, 1 w, 2 ζ, 1 z	–	–	18–22 N, 1 w, 2 ζ, 1 z	21–23 N, 1 w, 2 ζ, 1 z	23–24 N, 1 w, 2 ζ, 1 z	20–24 N, 1 w, 2 ζ, 1 z	23 N, 1 w, 2 ζ, 1 z	21–24 N, 1 w, 2 ζ, 1 z
Cx III	1 N	–	–	1 N	1 N	1 N	1 N	1 N	1 N
Tr III	1 N	–	–	1 N	1 N	1 N	1 N	1 N	1 N
bFe III	2 N	–	–	2 N	3 N	3 N	3 N	3 N	3 N
tFe III	5 N	–	–	5 N	5 N	5 N	5 N	5 N	5 N

Table 5 (continued)

Adults	<i>Erythraeus phalangoides</i>	<i>Erythraeus cinereus</i>	<i>Erythraeus regalis</i>
Ge III	8(5) N	8 N	8 N
Ti III	14–15 N, 1 φ	14–15 N, 1(2) φ	8 N(1 φ)
Ta III	18–23 N, 1 ζ	24(23) N, 1 ζ	15 N, 1 φ 22–26 N, 1 ζ
		8 N 15 N, 1 φ 20–23 N, 1 ζ	8 N 15 N, 1 φ 25 N, 1 ζ

For *E. phalangoides*, note the high number of conalae on palp genu (PaGe) and of normal setae (AL) on anterior sensillary area of crista metopica in adults as well as fewer setae on basifemora (bFe) I–III in larvae; opisthosoma chaetotaxy (NDV) includes pseudanal (*ps*) setae; character values given in parentheses apply to single observations

face of palp tibia and sometimes also on palp genu, ventrally, a row of cone-like, serrate setae (conalae), accompanied by more slender semi-conalae; leg segmentation formula 7-7-7-7; legs with modified stout setae—serratalae

Deutonymph: Similar to adult but smaller and with sparser setation; urvulva (external genital plate of deutonymph, after Southcott (1961)) of comparable size to anal sclerite or smaller; conalae and semi-conalae of palp genu and palp tibia, if present, more weakly developed (both, with respect to the number and size)

Larva (after Wohltmann et al. (2007); Southcott (1988)): Scutum oval or trapezoidal in outline, with two pairs of trichobothria and two pairs of non-sensillary setae; eyes, each composed of double lens, placed laterally to scutum; leg segmentation formula 7-7-7; $f_nCx = 1-1-1$, $f_nTr = 1-1-1$, and $f_nbFe = 2-2-2$ or $3-3-3$

Remarks: Southcott (1961), in the generic diagnosis of *Erythraeus*, listed the lack of serratalae on legs, contrary to their presence in the Australian *Erythroides* Southcott, 1946b; for the latter genus, the tentative, separate generic affiliation can be confirmed by the lack of conalae in active postlarval forms and presence of three pairs of scutal setae in larvae

Type species: *Acarus phalangoides* De Geer, 1778

Distribution: Worldwide

***Erythraeus phalangoides* (De Geer, 1778)**

Acarus phalangoides De Geer, 1778

Bochartia adrastus Southcott, 1961, syn. nov.

Type material: Neotype (NHRS-ACAR 000000095), male (for the place of origin, see Table 1, locality 5, collection date: 18 June 2011), mounted on one slide, is deposited in the Swedish Museum of Natural History, Stockholm, Sweden

Diagnosis (data for neotype given in parentheses)

Adult: Palp genu: 2–6 (2) conalae, 0–3 (1) semi-conalae; palp tibia: 3–6 (4) conalae, 0–1 (1) semi-conalae; anterior sensillary area of crista metopica (ASA) with indistinct anterior process and with 15–30 (20) non-sensillary setae; dorsal opisthosomal setae uniform, robust, cone-like, with stout setules; serratalae moderately developed, present on all legs (see Fig. 3 for comparison)

Larva: AL not inflated basally, ASens short (14–32), $fD = 34-36$, $f_nbFe = 2-2-2$, Ti III 145–200

Description

Adult: Fig. 3; metric data: Table 3; meristic data: Table 5

Color in life reddish-brown with black setae, eyes bright red

Gnathosoma: Chelicerae typical for the genus; palps with relatively sparse setation; normal, setulated setae present on palp trochanter and palp femur; palp genu with strong, serrate setae dorsally, smooth, and more slender setae laterally, two to

six conalae and zero to three spine-like semi-conalae (i.e., setae with rough, serrated surface, not more than twice as long as conalae, pointed); palp tibia with normal, sparsely barbed setae and with three to six conalae and zero to one semi-conalae; odontus hook-like; palp tarsus hemispherical, with the apical part overreaching the termination of odontus, densely covered with eupathidia and solenidia

Idiosoma: Scutum roughly limited to crista metopica; anterior process of ASA, weakly sclerotized, indistinct; posterior process of posterior sensillary area (PSA) absent; ASens and PSens covered with very tiny barbs; on ASA—15–30 setae AL; ocular plates (75–90 × 100–120), located laterally to crista, at ca half of its length; dorsal setae uniform in shape, short (22–70), cone-like, rounded at tip, covered with stout setules; ventral setae (VS) slender, covered with short setules; GOP surrounded by paired sclerites (genital plates: epivalves and

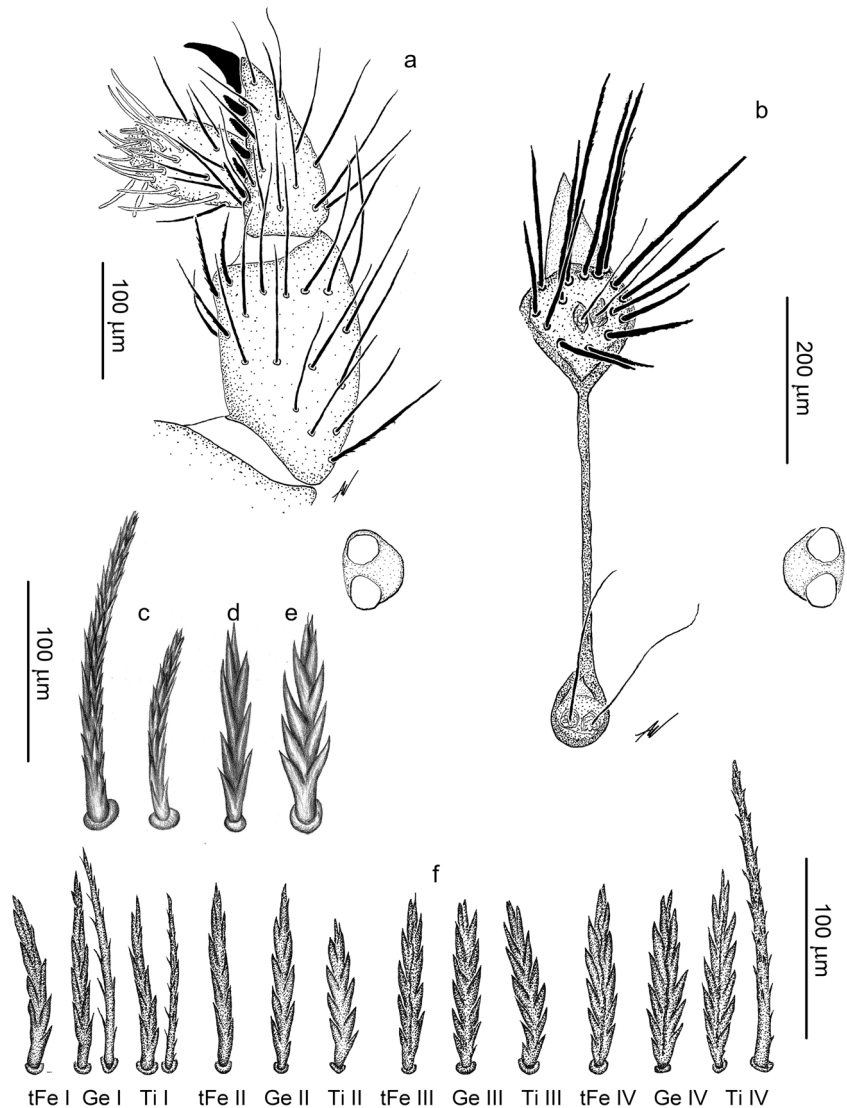
centrovalves), located at the level of coxae III–IV; setae covering valves shorter than VS, slender, with delicate barbs; AOP surrounded by indistinct paired valves, and of those, the external valves more sclerotized; setae covering valves similar to those covering genital plates but shorter

Legs: Serratae present on basifemora, telofemora, genua, and tibiae of all legs; the most stout ones occur on leg IV; other setae of normal shape; setulated setae present on all leg segments (on genua and tibiae—in minority); specialized setae distributed on bFe–Ta; tarsi I–IV terminated in paired claws, each claw covered with fimbriae

Larva: Figs. 4, 5, 6, and 7, habitus as in Fig. 4; metric data: Table 4; meristic data (including leg chaetotaxy): Table 5

Color in life light orange, with distinct yellowish rings on legs I–III

Fig. 8 *Erythraeus cinereus*, adult. **a** Palp, medial view. **b** Crista metopica and eyes. **c** Dorsal opisthosomal setae. **d** Serratala on genu I. **e** Serratala on genu IV. **f** Diversity of serratalae and setae of non-serratalae type on telofemora, genua, and tibiae of legs I–IV

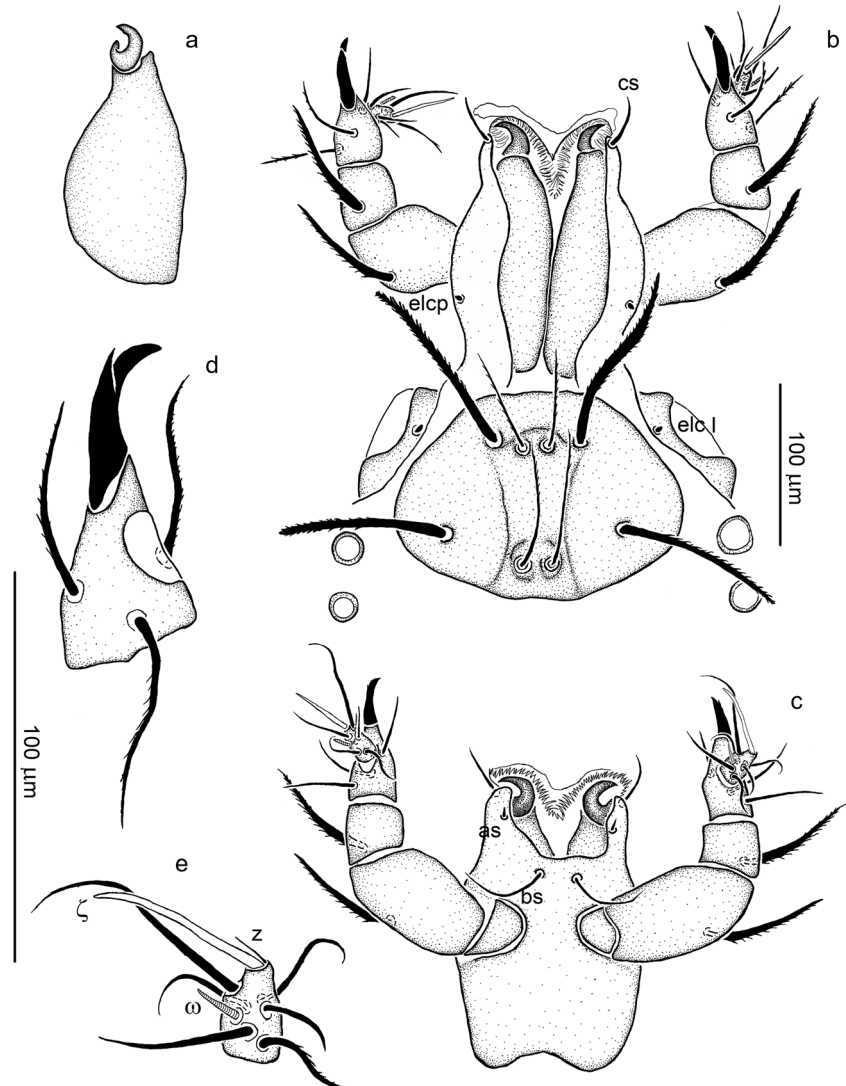


Gnathosoma (Fig. 5): Cheliceral claw, with distinct sub-terminal hook on the blade; a pair of smooth, pointed adoral setae (*cs*) (32–44), located distally; paired, club-shaped, supracoxal setae (*elcp*, 4–6) placed dorsally, in antero-lateral position; on ventral side of gnathosoma—a pair of smooth, pointed subcapitular (hypostomal, tritorstral) setae (*bs*) (50–70) and, in more anterior position—a pair of spine-like oral setae (*as*) (10–14); pedipalp formula: 0-B-B-BBB-NNNNNN $\omega\zeta z$; palp femur and genu, each with one setulated seta dorsally, palp femoral seta (40–50) always shorter than palp genual seta (60–80); palp tibia with three setae covered with indistinct setules; odontus with dorsal, splinter-like branch, arising at half length of claw and not reaching claw termination; palp tarsus with one distinctly elongated and five shorter, smooth normal setae, one solenidion (ω) and one prominent distal eupathidium (ζ); the latter associated with tiny companion seta (*z*)

Idiosoma (Fig. 6): Scutum oval in outline; anterior border almost straight, lateral, and posterior borders rounded; AL not widened at base; AL and PL serrate, ASens much shorter than PSens; paired eyes located postero-laterally of scutum, anterior lens (10–18) slightly larger than the posterior lens (8–16); dorsal setae short (25–60), thick, serrate; NDV (total number of setae on idiosoma, excl. setae on scutum and intercoxalae) = 42–52; coxa I with serrate seta 1b (80–100), seta 1a (71–104) located outside coxal plate I; coxa II with serrate seta 2b (25–38); coxa III with serrate seta 3b (28–40); seta 3a (28–38) located outside coxa III; posterior to coxae III four pseudanal setae, *ps* (24–55), distinctly thinner than dorsal idiosomal setae

Legs (Fig. 7): Segmentation formula: 7-7-7; solenidia on genu I and tibia I short (20–30); solenidion (σ) on genu located about half length of the segment, solenidia (φ) on tibia I located in distal half of the segment, distal solenidion with companala *da*; solenidion (ω) on

Fig. 9 *Erythraeus cinereus*, larva. **a** Chelicera. **b** Gnathosoma (and scutum), dorsal view. **c** Gnathosoma, ventral view. **d** Palp tibia. **e** Palp tarsus



tarsus I prominent (40–45), famulus (ϵ) (4–5) small, inconspicuous; subterminal eupathidium on tarsus I long (60–70) and with accompanying seta z (10–20), “pretarsal” eupathidium shorter (25–27), without z ; all solenidia on leg II short (15–20); solenidia on tibia II arise at proximal and distal margin of the segment, respectively; subterminal eupathidium on tarsus II long (40–50) and with companala z (7–15), “pretarsal” eupathidium shorter (20–25), without z . Solenidion on tibia III short (15–20), located proximally, “pretarsal” eupathidium short (20–25), without z ; median empodium of pretarsi I–III claw-like, with small lateral barbs; anterior claw with terminal hook, posterior claw without hook; both claws aliform

Distribution: Western Palaearctic

Remarks on taxonomy

Larvae of *E. phalangoides* fit the description of *E. adrastus* (Southcott, 1961). The specimens upon which Southcott (1961) based his description had been sampled in Denmark, on the sides of ant hills by Haarløv (1957), who reported the larvae to represent *E. phalangoides*. Southcott (1961) assumed that *E. adrastus* constitutes the larva of *E. phalangoides*; however, in the lack of correlation confirmed by rearing, he refrained from synonymization. Present laboratory rearing of *E. phalangoides* eggs, obtained from field-collected females, confirms the supposition of Haarløv (1957) and Southcott (1961); thus, *E. adrastus* should be regarded a junior synonym of *E. phalangoides*.

Biology

Field data and laboratory data are provided in Table 2. *E. phalangoides* is associated with a wide range of habitats, from sandy dunes close to marine

Fig. 10 *Erythraeus cinereus*, larva. **a** Gnathosoma and idiosoma, dorsal view. **b** Dorsal opisthosomal setae. **c** Gnathosoma and idiosoma, ventral view. **d** Seta *ps*

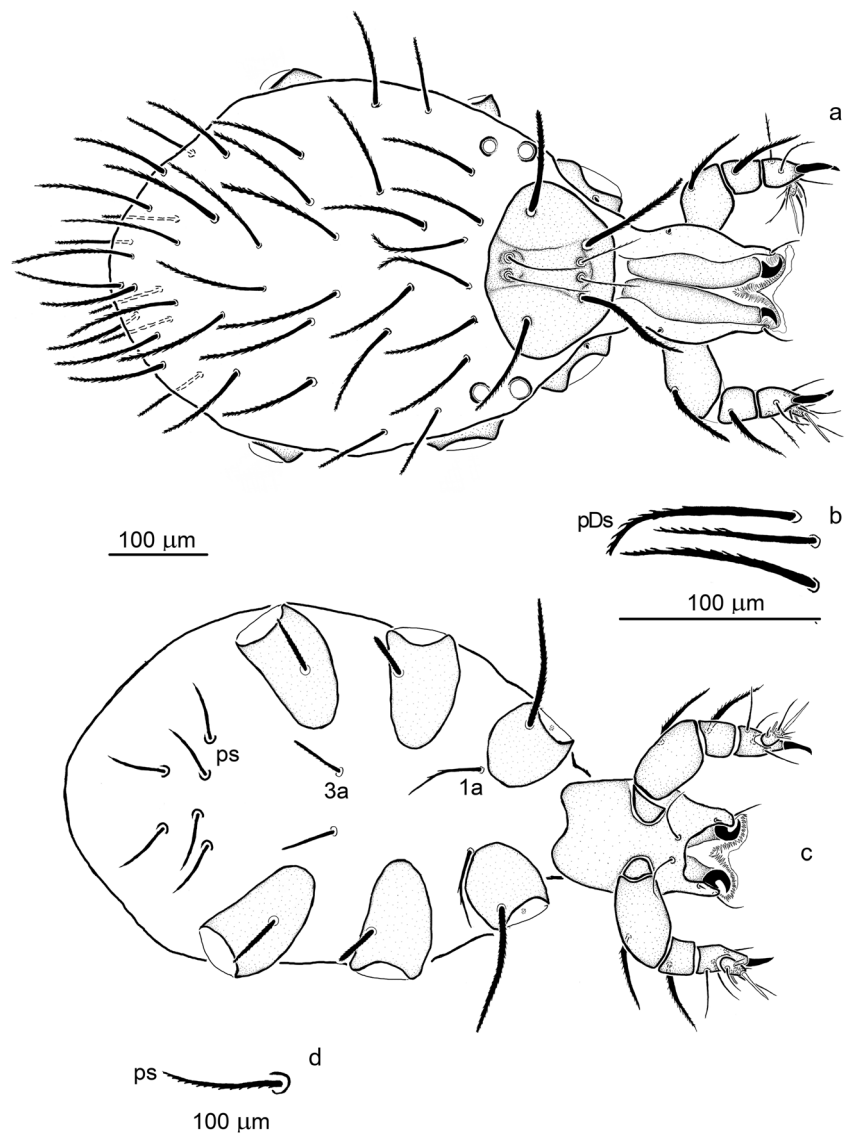
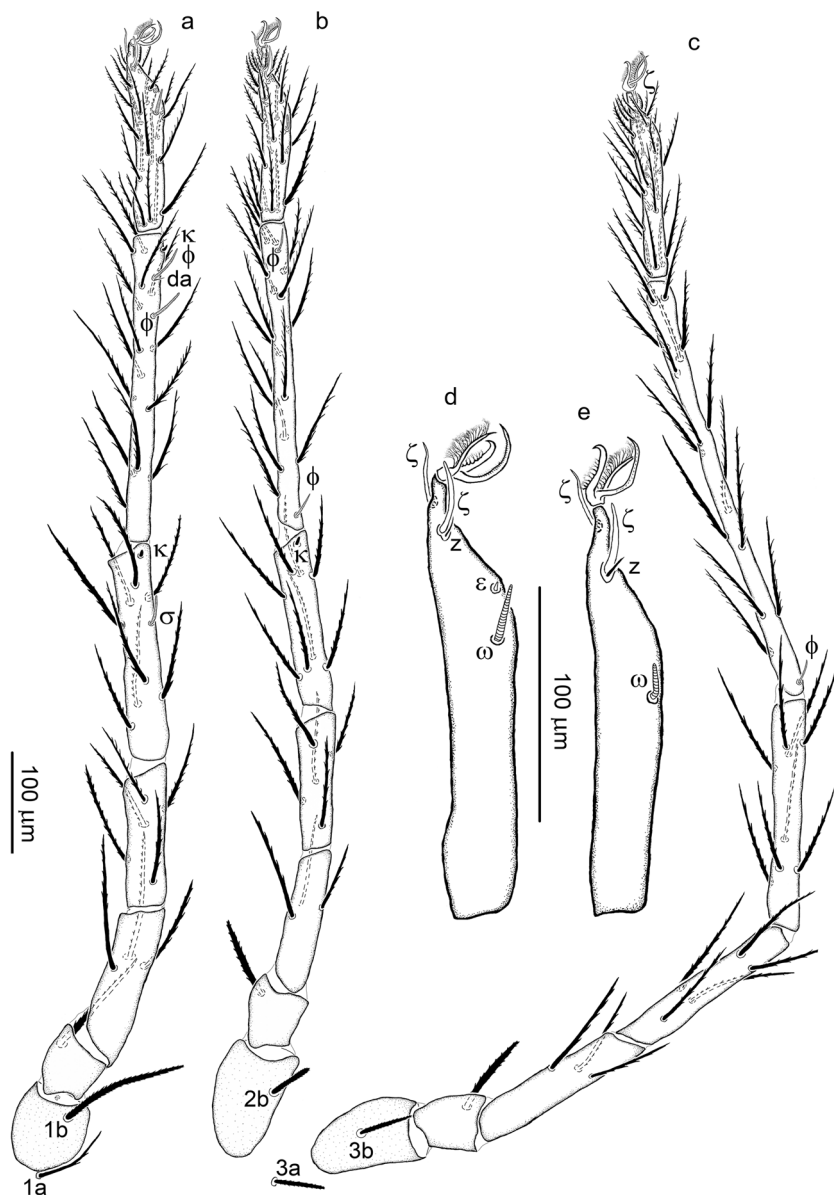


Fig. 11 *Erythraeus cinereus*, larva. **a** Leg I. **b** Leg II. **c** Leg III. **d** Tarsus I. **e** Tarsus II (**d**, **e**, only specialized setae shown)



coasts over forested biotopes in lowlands to xeric meadows. In late May to July, deutonymphs and adults were observed to move quickly on the surface of litter layer and to feed on adult ants. Moreover, a single case of preying of adult *E. phalangoides* on a small beetle was observed. Larvae parasitized aphids in the field and in the laboratory.

During laboratory experiments, eggs underwent further development only after obligatory chilling, which provided a basis to consider the egg a diapausing and hibernating instar. In parasitism experiments, it was observed that unfed larvae attached to conspecifics which already had fed on a host. Such behavior was not observed when unfed larvae were kept together, without aphids. Spermatophore deposition was observed short time after collection in June. Spermatophores were

deposited at subsaturated humidity conditions, only in the manner described by Witte (1991). Each spermatophore consisted of a whitish stalk (about 1200 μm) and a spherical sperm droplet (about 440 μm).

***E. cinereus* (Dugès, 1834)**

Rhyncholophus cinereus Dugès, 1834

Erythraeus jowitae Haitlinger, 1987, syn. nov.

Erythraeus sp.: Wohltmann 1998

Erythraeus sp. A.: Wohltmann (2001)

Type material: Neotype (NHRS-ACAR 000000096), male (for the place of origin, see Table 1, locality 15, collection date: 28 July 1997), mounted on one slide, is deposited in the Swedish Museum of Natural History, Stockholm, Sweden

Diagnosis (data for neotype given in parentheses)

Adult: Palp genu: 0–4 (2) semi-conalae; palp tibia: 4–6 conalae (5), 0–1 (0) semi-conalae; anterior sensillary area of crista metopica with anterior process and with 7–15 (11) non-sensillary setae; dorsal opisthosomal setae differentiated into two types, either straight or slightly bent, with husk-like setules; longer setae parallel sided or slightly narrowing apically, shorter—more distinctly narrowing towards termination; serratae present on all legs, the most robust ones—on leg IV (see Fig. 8 for comparison)

Larva: AL not inflated basally, ASens long (60–90), fD=34–44, f_nbFe=3-3-3, Ti III 416–485

Description

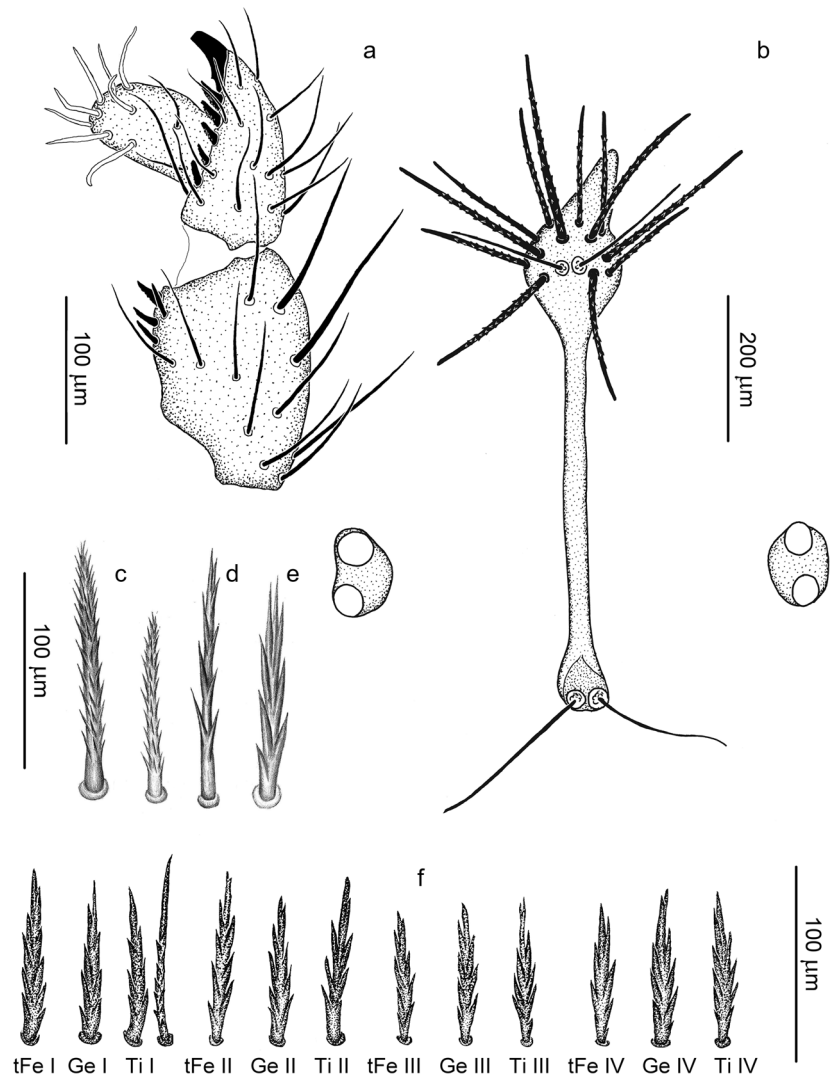
Adult: Fig. 8; metric data: Table 3; meristic data: Table 5
Color in life reddish-brown, eyes bright red

Gnathosoma: Chelicerae typical for the genus; palps with relatively sparse setation; normal, setulated setae present on palp trochanter and palp femur; palp genu

with strong, serrate setae dorsally, smooth and more slender setae laterally, zero to four semi-conalae (i.e. setae with rough, serrated surface, not more than twice as long as conalae, pointed); conalae absent from palp genu; palp tibia with normal, sparsely barbed setae and with four to six conalae and zero to one semi-conalae; odontus hook-like; palp tarsus hemispherical, with the apical part overreaching the termination of odontus, densely covered with eupathidia and solenidia

Idiosoma: Scutum roughly limited to crista metopica; anterior process of ASA distinct, posterior process of PSA absent; ASens and PSens covered with very tiny barbs; on ASA, 7–15 setae AL; ocular plates (70–80 × 100–125), located laterally to crista, at about half of its length; dorsal setae (ca 40–215) of two types (the most striking differences apply to the extreme forms); longer setae parallel sided or slightly narrowing apically, shorter—more distinctly narrowing towards termination, all covered with relatively stout setules;

Fig. 12 *Erythraeus regalis*, adult. **a** Palp, medial view. **b** Crista metopica and eyes. **c** Dorsal opisthosomal setae. **d** Serratala on genu I. **e** Serratala on genu IV. **f** Diversity of serratalae and setae of non-serratalae type on telofemora, genua, and tibiae of legs I–IV



ventral setae much more slender than dorsal ones, covered with short setules. GOP surrounded by paired sclerites (genital plates: epivalves and centrovalves), located at the level of coxae III–IV; setae covering valves shorter than VS, slender, with delicate barbs; AOP surrounded by indistinct paired valves, and of those, the external valves are more sclerotized; setae covering valves similar to those covering genital plates but shorter

Legs: Serratae present on basifemora, telofemora, genua, and tibiae of all legs; on legs I–II, more weakly developed than on legs III–IV; the most stout serratae occur on tibia IV; of other setae—normal, setulated ones, present on all leg segments (on genua and tibiae—in minority); specialized setae distributed on bFe–Ta; Tarsi I–IV terminated in paired claws, each claw covered with fimbriae

Larva: Figures 9, 10, and 11; metric data: Table 4; meristic data (including leg chaetotaxy): Table 5

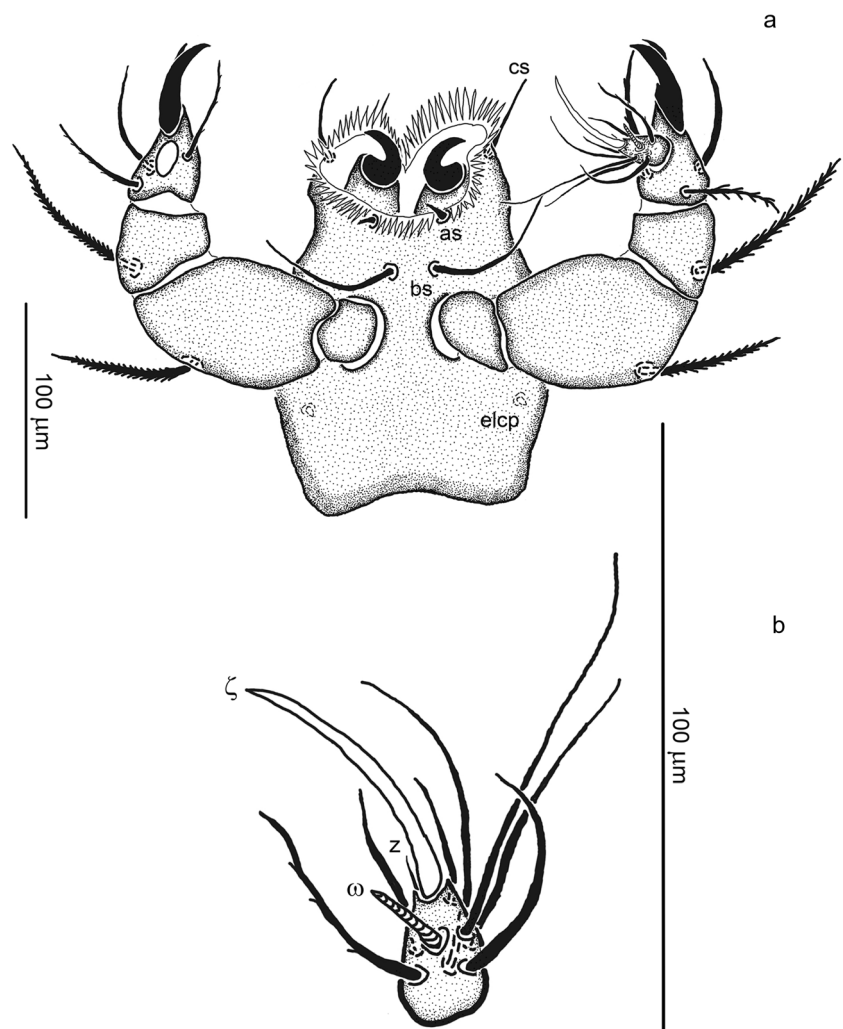
Color in life orange to reddish

Gnathosoma (Fig. 9): Cheliceral claw, with distinct subterminal hook on the blade; a pair of smooth, pointed adoral setae

(*cs*) (31–48), located distally; paired, club-shaped, supracoxal setae (*elcp*, 4–6) placed dorsally, in antero-lateral position; on ventral side of gnathosoma—a pair of smooth, pointed subcapitular (hypostomal, tritorstral) setae (*bs*) (50–74) and, in more distal position, a pair of spine-like oral setae (*as*) (8–16); pedipalp formula: 0-B-B-BBB-BNNNNN ω ζ ; palp femur and genu, each with one setulated seta dorsally, palpal femoral seta (70–87) of similar length as palpal genual seta (70–95); setae on palp tibia covered with indistinct setules; odontus with dorsal, splinter-like branch, arising at half length of claw and not reaching claw termination; palp tarsus with one distinctly longer and five shorter, normal setae, one solenidion (ω) and one prominent distal eupathidium (ζ), the latter associated with tiny companion seta (*z*)

Idiosoma (Fig. 10): Scutum hexagonal in outline, widening postero-laterally; anterior border almost straight, lateral and posterior borders rounded; AL not widened at base, both AL and PL serrate; ASens slightly shorter than PSens; paired eyes located postero-laterally of scutum, anterior lens (22) slightly larger

Fig. 13 *Erythraeus regalis*, larva. **a** Gnathosoma, ventral view. **b** Palp tarsus



than the posterior lens (20); dorsal setae variable in length (59–143), serrate; NDV=46–58; coxa I with serrate seta 1b (120–140), seta 1a (70–95) located outside coxal plate I; coxa II with serrate seta 2b (40–60) (in one larva setae 2b extraordinarily long, 100–105); coxa III with serrate seta 3b (48–68); seta 3a (42–56) located outside coxa III; posterior to coxae III six pseudanal setae (55–60), distinctly thinner than dorsal idiosomal setae

Legs (Fig. 11): Segmentation formula: 7-7-7; solenidion on genu I (33–38) and solenidia on tibia I (30–40) of similar length; solenidion on genu (σ) arising at ca half length of the segment; both solenidia on tibia (φ) located in distal half of the segment, distal solenidion with companala *da*; solenidion (ω) on tarsus I relatively short (25–32), famulus (ϵ) (4–5) small, inconspicuous; subterminal eupathidium on tarsus I

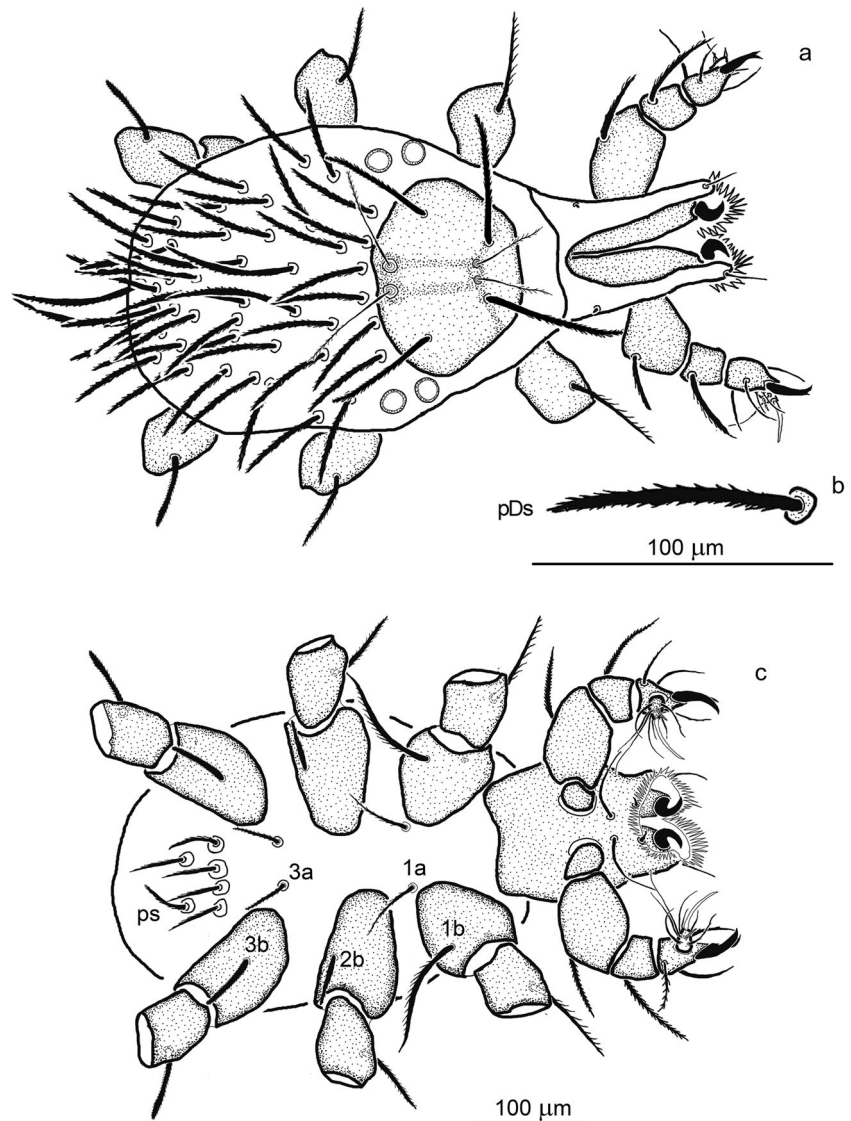
also relatively short (30–40) and with accompanying seta *z* (8–12), “pretarsal” eupathidium shorter (24–30), without *z*; all solenidia on leg II moderately short (20–30); solenidia on tibia II arise at proximal and distal margin of the segment, respectively; subterminal eupathidium on tarsus II (35–40) with companala *z* (7–10), “pretarsal” eupathidium shorter (21–28), without *z*; solenidion on tibia III short (21–25), located proximally, “pretarsal” eupathidium (25–32), without *z*; median empodium of pretarsi I–III claw-like, with small lateral barbs; anterior claw with terminal hook, posterior claw without hook; both claws aliform

Distribution: Western Palaearctic

Remarks on taxonomy

Considering the set of morphological traits but also the ecological preferences and frequency of occurrence, larvae of *E. cinereus* match *E. jowitzae* Haitlinger, 1987. The latter

Fig. 14 *Erythraeus regalis*, larva. **a** Gnathosoma and idiosoma, dorsal view. **b** Dorsal opisthosomal seta. **c** Gnathosoma and idiosoma, ventral view



species was described based on two specimens and since then has been recorded from several European countries (see distribution). Here, it is considered as junior synonym of *E. cinereus*.

Biology

Field data and laboratory data are provided in Table 2. *E. cinereus* was collected at forests, forest margins, at flooded valleys and the reed belts on the ponds, at one locality part of the habitat was exposed to annual flooding by a nearby creek in winter and in spring. The maximum abundance of larvae was observed in July. Deutonymphs were found in litter layer. Adults were active at sunny days in August and September but remained motionless preferably at the underside of rotting leaves when temperatures were below 10 °C. In January and February, diapausing adults were sometimes found frozen but became active when exposed to 20 °C in the laboratory. In the field, adults became active again in spring, when they searched the surface of the litter layer for prey and mates.

In the laboratory, only adults collected in spring deposited eggs ($n=31$), while females collected in autumn or winter never did so ($n=40$). *E. cinereus* eggs remained viable at 76–100 % relative air humidity and survived even permanent submersion in tap water. Partially fed larvae became attractive as host to unfed conspecifics, which in turn started parasitism. These larvae could again become attractive to other unfed larvae, and in several cases, chains of up to four *E. cinereus* larvae parasitizing each other were found, which votes for the phenomenon of hyperparasitism existing in *Erythraeus* spp. In a few cases, both the larva parasitizing the aphid host as well as another larva parasitizing its conspecific reached the next life instar. However, in majority of cases, the uptake of nutrients from conspecifics was not sufficient and, after detachment, larvae continued to display host searching behavior, sometimes leaving larvae dead and sucked empty. Parasitism on unfed larvae of the same species was observed exceptionally ($n=3$), 7 days after hatching, and none of the parasitic larvae reached the subsequent life instar. Larvae of ants, nymphs, and adults of plant bugs were not accepted by *E. cinereus* larvae as potential hosts. Deutonymphs and adults fed on larvae and pupae of ants and brachyceran flies. Adults kept at constant 20 °C moved around and fed on prey; however, neither spermatophores nor eggs were deposited. Exposure to 5 °C for 71 days again did not induce reproductive behavior after re-exposition to 20 °C ($n=15$). However, when chilled for 105 days, males deposited spermatophores 5–7 days after re-exposition to 20 °C ($n=3$), which points at adult as an hibernating instar.

E. regalis (C.L. Koch, 1837)

Rhyncholophus regalis C.L. Koch, 1837

Bochartia kuyperi Oudemans, 1910, syn. nov.

Erythraeus gertrudae Haitlinger, 1987, syn. nov.

Erythraeus sp. A.: Wendt (1996)

Erythraeus nivalis (sic!): Wohltmann (2001)

Type material: Neotype (NHRS-ACAR 000000097), female (for the place of origin, see Table 1, locality 23, collection date: 24 May 2015), mounted on one slide, is deposited in the Swedish Museum of Natural History, Stockholm, Sweden.

Diagnosis (data for neotype given in parentheses)

Adult: Palp genu: 0–3 (2) conalae, 0–2 (0) semi-conalae; palp tibia: 2–5 (5) conalae, 0–2 (0) semi-conalae; anterior sensillary area of crista metopica with anterior process and with 8–12 (12) non-sensillary setae; dorsal opisthosomal setae uniform in shape, straight or only slightly bent, narrowing apically, with slender, husk-like setules; serratae on legs I–II slender, on legs III–IV moderately developed (see Fig. 12 for comparison)

Larva: AL not inflated basally, ASens relatively long (50–76), fD=30–42, f_nbFe=3-3-3, Ti III 310–385

Description

Adult: Fig. 12; metric data: Table 3; meristic data: Table 5
Color in life reddish-brown

Gnathosoma: Chelicerae typical for the genus; palps with relatively sparse setation; normal, setulated setae present on palp trochanter and palp femur; palp genu with almost smooth, slender setae (slightly thicker at dorsal side of the segment), zero to three conalae and zero to two semi-conalae; palp tibia with normal, sparsely barbed setae and with two to five conalae and zero to two semi-conalae; odontus hook-like; palp tarsus rounded at termination, with the apical part overreaching the termination of odontus, densely covered with eupathidia and solenidia

Idiosoma: Scutum indistinct, not extended beyond crista metopica; anterior process of ASA relatively narrow, posterior process of PSA absent; ASens and PSens covered with very tiny barbs; on ASA—8–12 setae AL; ocular plates (73–88 × 108–127), located laterally to crista, behind the half of its length; dorsal setae (50–175) uniform in shape, only slightly narrowing apically, densely covered with husk-like setules (more delicate than in *E. cinereus*); setules adnate in proximal part of the stem, become more outstanding towards stem termination; ventral setae much more slender than dorsal ones, needle-like, covered with short bristles; GOP surrounded by paired sclerites (genital plates: epivalves and centrovalves), located at the level of coxae III–IV; setae covering valves similar to VS, slender, with delicate barbs; AOP surrounded by indistinct paired valves, and of those, the external valves were more sclerotized; setae covering valves similar to those covering genital plates but shorter

Legs: Serratae present on basifemora, telofemora, genua, and tibiae of all legs; on legs I–II serratae slender than on

legs III–IV; the most stout serratae occur on tibia IV; of other setae—normal, setulated ones, present on all leg segments (on genua and tibiae—in minority); specialized setae distributed on bFe–Ta; tarsi I–IV terminated in paired claws, each claw covered with fimbriae

Larva: Figs. 13, 14, 15, and 16; metric data: Table 4; meristic data (including leg chaetotaxy): Table 5

Color in life bright red

Gnathosoma (Fig. 13): Cheliceral claw, with distinct subterminal hook on the blade; a pair of smooth, pointed adoral setae (*cs*) (33–46), located distally; paired, club-shaped, supracoxal setae (*elcp*, 5–7) placed dorsally, in antero-lateral position; on ventral side of gnathosoma, a pair of smooth, pointed subcapitular (hypostomal, tritorstral) setae (*bs*) (56–69) and, in more distal position, a pair of spine-like oral setae (*as*) (6–15); pedipalp formula: 0-B-B-BBB-BNNNNN $\omega\zeta z$; palp femur and genu, each with one setulated seta dorsally, palpal femoral seta (60–71), slightly shorter than palpal genual seta (79–91); the most proximal seta on palp tibia with short setules, the most distally placed seta (in ventral position) almost smooth; odontus with dorsal, splinter-like branch, arising at half length of claw and not reaching claw termination; palp tarsus with one elongated, barbed seta, and five to six shorter, normal setae (two—with minute setules), one solenidion (ω) and one prominent distal eupathidium (ζ), the latter associated with tiny companion seta (*z*)

Idiosoma (Figs. 14 and 15): Scutum oval to trapezoidal in shape; AL not widened at base, both AL and PL serrate;

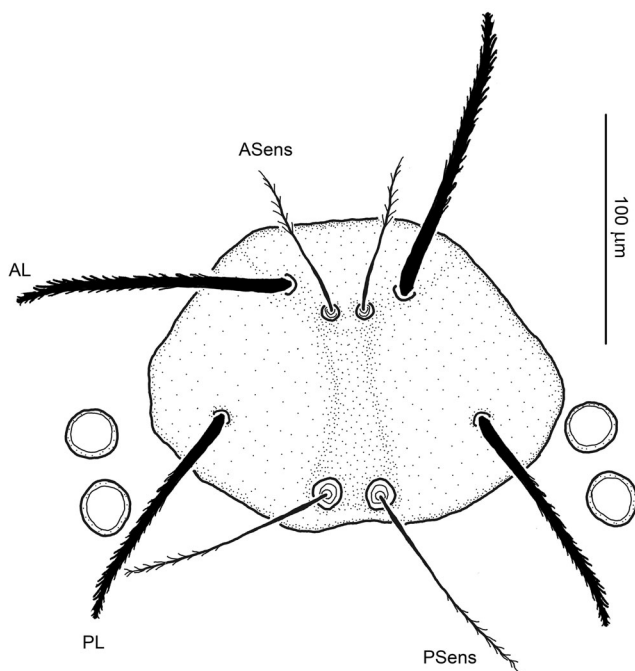


Fig. 15 *Erythraeus regalis*, larva. Scutum

ASens shorter than PSens; paired eyes located postero-laterally of scutum, at the level of posterior margin of the sclerite (in unengorged specimens); anterior lens (18–19) and posterior lens (17–19) comparable in size; dorsal setae variable in length (58–127), serrate; NDV=42–52; seta 1a (60–70) located outside coxal plate I; coxa I with serrate seta 1b (106–120), coxa II with serrate seta 2b (43–44), coxa III with serrate seta 3b (49–53); seta 3a (38–43) located outside coxa III; posterior to coxae III six pseudanal setae (50–58), distinctly thinner than dorsal idiosomal setae

Legs (Fig. 16): Segmentation formula: 7-7-7; solenidion on genu I (25–27) located at ca half length of the segment; proximal solenidion on tibia I (28–30) slightly shorter than the distal solenidion (32–36); both solenidia on tibia located in distal half of the segment, distal solenidion with companala *da*; solenidion (ω) on tarsus I relatively short (24–28), famulus (ϵ) (3) small, inconspicuous; subterminal eupathidium on tarsus I also relatively short (32–35) and with accompanying seta *z* (9), “pretarsal” eupathidium markedly shorter (17–20), without *z*; solenidia on tibia II arise at proximal and distal margin of the segment, respectively; proximal solenidion (21–24) longer than the distal one (15); subterminal eupathidium on tarsus II (34–38) with companala *z* (9), “pretarsal” eupathidium shorter (23–25), without *z*; solenidion on tibia III short (22–25), located proximally, “pretarsal” eupathidium (19–27), without *z*; median empodium of pretarsi I–III claw-like, with small lateral barbs; anterior claw with terminal hook, posterior claw without terminal hook; both claws aliform

Distribution: Western Palaearctic

Remarks on taxonomy

E. kuyperi and *E. gertrudae* fall within the range of morphometric characters as measured for larvae of *E. regalis*. Haitlinger (2003), in the key to larvae of *Erythraeus* spp., pointed to differences between *E. kuyperi* and *E. gertrudae* expressed in the shape of scutum (oval in *E. kuyperi*, trapezoidal in *E. gertrudae*). Examination of larvae obtained by experimental rearing allowed to attribute the differences to intraspecific variation, thus constitutes the background for species synonymization.

Biology

Field data and laboratory data are provided in Table 2. *E. regalis* was collected close to water reservoirs, both in meadows and in forested areas, however not at inundated places. A single protonymph was collected in the litter layer in August. Deutonymphs were active at sunny days in August–September and again in March moved around on the litter layer. Outside the time of increased activity, they hid in crevices of the bark of *Betula* 1–3 m above ground level. In May 16, tritonymphs were found in crevices, most in the bark of birch trees and only few in the litter layer. Adults were active in May–June.

Fig. 16 *Erythraeus regalis*, larva. **a** Leg I. **b** Leg II. **c** Leg III. **d** Genu-tarsus I. **e** Genu-tarsus II. Tibia-tarsus III (**d–f**, only specialized setae shown)



In the laboratory, eggs were deposited by females collected in early summer. Larvae hatched a month later and survived for up to 13 days without hosts ($n = 60$, 20 °C). When exposed to *Aphis* sp., larvae attached to the host at first contact. Further development stopped when reaching the deutonymph instar. Although deutonymphs moved and fed on larvae and pupae of ants and brachyceran flies, none entered the tritonymph when kept at constant 20 °C. However, of ten deutonymphs captured in September and chilled at 5 °C and darkness for 102 days, one entered the tritonymph when subsequently exposed to 20 °C and 16/8 light/darkness. The latter constitutes an evidence for deutonymph being a diapausing and hibernating instar.

Concluding remarks

For *E. regalis* and *E. cinereus*, the results of the molecular analysis were confirmed by laboratory rearing. In

case of *E. phalangoides*, matching of larvae and active postlarval forms was based on the results of experimental rearing, whereas the distinct ID of the species was confirmed by molecular data. Both molecular analyses and experimental rearing allowed for unambiguous correlation of heteromorphic instars and, in consequence, subsequent morphological investigations of specimens collected at different geographical areas.

The correlation of heteromorphic, metamorphosed instars through DNA analysis and laboratory rearing has been infrequently applied up to date in relation to various non-parasitengone arthropods (Grutter et al. 2000; Barros-Battesti et al. 2007; Kumar et al. 2007; González et al. 2015). As application of both methods led to the same results, we are convinced that DNA barcoding is a highly cost-effective alternative or complement to experimental rearing practice, especially when it comes to problematic species (Hebert et al. 2003; Kress et al. 2015). The plethora of studies now using solely DNA-

based species identification to link life history stages and to study trophic interactions is a convincing testimony (see references in “Introduction”). In both cases, the sampling effort is comparable, however, with slightly different approach. While the collecting of active postlarval forms for the purpose of molecular delimitation does not need to focus on ovigerous females, the arthropod-parasitizing larvae require complementary collecting methods.

Although rearing gives a vital insight in the species biology, the disadvantage is the time. The faster genetic matching has its flaws in laboratory costs and requires access to molecular facilities. On the other hand, the per base pair sequencing cost has plummeted in the last 10 years, thanks to high-throughput Sanger sequencing workflows as well as next-generation sequencing techniques. DNA-based species identification also has a number of challenges in the need of a representative reference library and can be misled by hybridization, introgression, incomplete lineage sorting, or young species radiation (see “Introduction”). Species delimitation using the GMYC model can be sensitive to low sampling, both at population level and at taxonomic scale (Lohse 2009). In our case, the inclusion of additional species of the same genus resulted in increased support in the gene tree and gave better performance in the species delimitation (narrowing the confidence interval). Also the possibility of contamination (gut content from host/prey) is harder to detect with fewer samples. As we observed, conspecific parasitism under laboratory conditions, it is not impossible that parasitism can occur in the wild between closely related species. DNA contamination from gut contents in such case could be very difficult to detect. Of several molecular species delimitation models, the GMYC model has the advantage of not requiring any predefined units. It is a so-called discovery method of species delimitation in contrast to validation methods that require a predefined hypothesis of species limits (Ence and Carstens 2011; Carstens et al. 2013). In this study, the combination of experimental rearing and molecular matching contributed to the knowledge of intraspecific variation of metric data and strengthened the conclusions resulting in formal synonymization of several nominal species names. This is a first step on the way towards resolving the double classification in *Erythraeus* and other parasitengone mites. As stated by de Queiroz (2007), assembling morphological, biological, and molecular data, gives multiple lines of evidence to species hypotheses in a unified species concept framework.

Acknowledgments We are grateful to all collectors who helped to obtain the specimens for studies. The staff at the Molecular Systematics Laboratory (MSL, NRM) is acknowledged for their valuable advice on sequencing. Our thanks go to D. Łupicki for his assistance in processing the original drawings of setae in postlarval forms of *Erythraeus* spp. JS and JB were supported (grant dha 154/09 1.4) by the Swedish Taxonomy Initiative.

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