Systematic Entemology

Systematic Entomology (2008), **33**, 548–551 DOI: 10.1111/j.1365-3113.2008.00420.x

What's in a frog stomach? Solving a 150-year-old mystery (Diptera: Calliphoridae)

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Abstract. The nominal taxon *Acanthosoma chrysalis* Mayer, 1844 is revised, and a lectotype is designated. The species, which was described from Germany from a number of alleged parasites encysted in the peritoneal wall of the stomach of edible frogs, is shown to be based on first instar larvae of blow flies (Calliphoridae). Argued from the shape and configuration of mouthhooks and abdominal cuticular spines, *Acanthosoma* Mayer, 1844 is shown to be a junior synonym of *Onesia* Robineau-Desvoidy, 1830, **syn.n.**, and *A. chrysalis* is shown to be a junior synonym of *O. floralis* Robineau-Desvoidy, 1830, **syn.n.** This species is an obligate parasitoid of earthworms, and it is hypothesized that first instar larvae enter the frogs through infected earthworms.

Introduction

More than 150 years ago, the German parasitologist Professor August Franz Joseph Carl Mayer made an unusual observation when he found small worm-like creatures thought to be parasites embedded in the peritoneal side of the stomach wall of the edible frogs he was dissecting (Mayer, 1844a, b). Three out of five dissected frogs were infested, and from four to ten of the assumed parasites were present. Mayer described and illustrated the 12 spiny rings of the body and what he tentatively considered to be a complex genital apparatus, and, as he had never encountered a similar creature or heard of the existence of anything like it, he gave it a scientific name. In recognition of the distinctively spiny body and his conviction that he was observing individuals of impending metamorphosis, Mayer named the creature *Acanthosoma chrysalis.* Perplexed by its presence only in the outer part of the stomach wall, that is, towards the body cavity, he classified the organism as an 'intermediate between the true entozoans and the parasitic insects' (Mayer, 1844b: '. . . in der Mitte stehen zwischen den eigentlichen Entozoen und den parasitischen Insekten').

Both the genus-group name *Acanthosoma* and the species-group name *chrysalis* were proposed by Mayer (1844a) in a short paper without illustration, which was followed later the same year by an equally short paper but with a slightly more detailed description and several illustrations (Mayer, 1844b). The nominal species *Acanthosoma chrysalis* was listed by Sherborn (1902) in his *Index Animalium* as a worm ('Verm.'), and later indexed by Neave (1939) in his *Nomenclator Zoologicus* as a dipteran larva. The only other appearance of the name that we have found is by Walton (1964), who compiled a list of insect parasites found in amphibians. Apparently, Mayer's discovery and observations never caught the attention of either parasitologists or entomologists, and they have largely faded into oblivion. We want to call attention to Mayer's observations for two reasons. Current nomenclatural practice bestows priority to zoological names according to their relative appearance in time (ICZN, 1999). Therefore, it becomes more than a pedantic exercise to provide the best taxonomic decisions on those nominal species that were proposed in the early literature and for which the type material may have been lost. [Actually, the name *Acanthosoma chrysalis* Mayer, 1844a was 'rediscovered' during an ongoing compilation of a nomenclatural authority file for Diptera (Evenhuis *et al.*, 2007), and its identity as a larva of a cyclorrhaphan Diptera was soon realized, which led to the question of taxonomic identity.] Moreover, in addition to its importance as a historical legacy, Mayer's discovery carries valuable biological information in its own right.

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Taxonomy

Mayer worked on recently killed frogs, and it is noteworthy that he stated that the parasitic creatures from the stomach wall were in a 'dormant-like stage' (Mayer, 1844a: '... schlummerähnliches Zustand...') and showed 'hardly detectable movements' (Mayer, 1844b: '... kaum merkliche Bewegung...'). Mayer also noted that, under the integument or 'skin' of an individual parasite, another thin integument could be observed, and as he furthermore found the individuals to be contained in an 'egg-shaped sheath' (Mayer, 1844b: '... eiförmige Umhüllung...'), he considered this to be evidence of incipient transformation or metamorphosis (Mayer, 1844b: 'Ohne Zweifel befindet sich das Entozoon noch im Zustande seiner Metamorphose...'). Although Mayer clearly was aware of the affinities to insects, he apparently did not see the incongruity of suggesting a well-developed copulatory organ in a pre-imaginal life stage; otherwise, he would never have made the bold assumption that the strongly sclerotized apparatus he could observe at one end of each specimen was a penis equipped with a pair of hooks (Mayer, 1844b; '... ein doppelhackiges Gebilde an einem Ende, welche ich für den Penis halten möchte.'). Owing to the immobility of the individuals, Mayer simply got the orientation wrong, and never realised that what he had in front of him was the first instar maggot of a dipteran, and what he considered to be a copulatory apparatus was actually the larval cephaloskeleton with its associated pair of mouthhooks.

Mayer (1844a, b) explicitly noted the 12 segments (spine-rings or 'Ringe von Stacheln'), which correspond to the pseudocephalon, three thoracic and seven abdominal segments, and a composite anal division of a schizophoran cyclorrhaphous larva. The figures provided by Mayer (1844b) furthermore show the distribution of spines on the larval body, the shape of the individual spines, and the distal part of the cephaloskeleton with paired mouthhooks (Fig. 1). These figures are sufficiently detailed to provide the evidence needed for a reliable identification, or at least to give a 'best match' against our current knowledge of European flies. The strong mouthhooks and the robust spines set in narrow rings anteriorly on each segment provide strong evidence that the larva belongs to either the Sarcophagidae or the Calliphoridae. An affiliation within the former, which in the present case largely would mean the flesh fly genus *Sarcophaga* (sensu lato), is eliminated by the shape of the anal division, which in *Acanthosoma* is smoothly rounded in profile rather than abruptly truncated as in *Sarcophaga*, and apparently without the spiracular depression so typical of flesh fly larvae.

The association with frogs would seem to hint at the genus *Lucilia* Robineau-Desvoidy, as two European species are known to produce myiasis in anuran amphibians: the obligate parasite *L. bufonivora* Moniez and the facultative parasite *L. silvarum* (Meigen) (Rognes, 1991; Zavadil *et al.*, 1997). Eggs are laid on the back of potential hosts, and when the anuran sheds its skin, the moisture induces the eggs to hatch, and the emerging first instar larvae are dragged forwards to the head as the host eats its own skin (Zumpt, 1965). Although it would seem at least possible that a few larvae are ingested with the skin and that the host escapes attack from the remaining larvae, the larval morphology falsifies such a hypothesis. In first instar *Lucilia*, a labrum is present, the mouthhooks take the form of elongated, stick-like structures, with the tip bending downwards at approximately a right angle, and they are equipped with two to three small teeth apically (Fig. 2A). The cuticular spines are straight or only slightly curved, not particularly elongated, and with the base not particularly broad (Fig. 2B). This is very different from the situation in *Acanthosoma*, where a labrum is absent, the mouthhooks are depicted as robust, almost straight, and with a simple tip, and the cuticular spines are elongate and with a broad, oval base (Fig. 1). Some other European genera of Calliphoridae can be ruled out by similar arguments: first instar larvae of *Calliphora* Robineau-Desvoidy are similar to *Lucilia* spp., but the mouthhooks have four to six teeth at their tip (Keilin, 1915); species of *Pollenia* Robineau-Desvoidy have sharp, pointed cuticular spines on the abdominal segments, and the shape of the mouthhooks differs markedly from that of saprophagous species by being less sclerotized and having from a few to several tiny spinules at the tip (Szpila, 2003). However, first

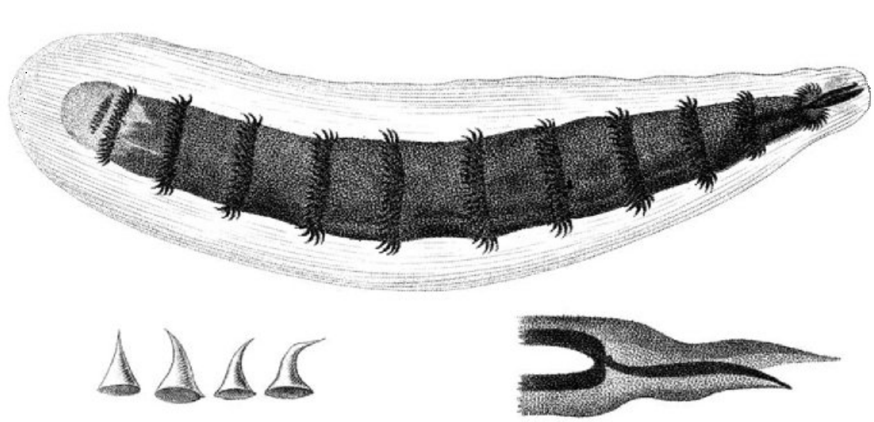

Fig. 1. *Acanthosoma chrysalis*. Mayer's original figure of *A. chrysalis* within its cyst, with insets showing enlarged cuticular spines (left) and paired mouthhooks (right).

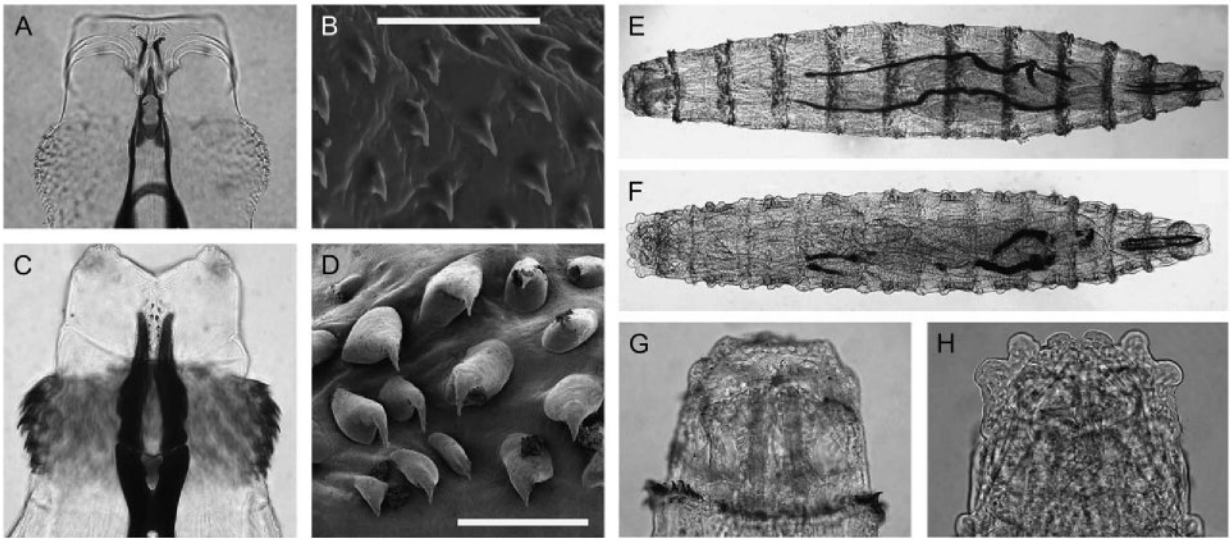

Fig. 2. Morphology of first instars of *Lucilia silvarum* (A, B), *Onesia floralis* (C, D, E, G), and *Bellardia vulgaris* (F, H). A, C. Pseudocephalon, ventral view. B, D. Abdominal cuticular spines (SEM; scale: 30 μm). E, F. Habitus, dorsal view. G, H. Anal division, dorsal view.

instar larvae of the earthworm-parasitizing blow flies of the genera *Bellardia* Robineau-Desvoidy and *Onesia* Robineau-Desvoidy are equipped with a single-tipped mouthhook and the labrum is reduced and without a projecting tip, thereby presenting a much better match. Recent morphological work on *Bellardia* and *Onesia* provides strong evidence that *Acanthosoma chrysalis* is conspecific with *Onesia floralis* Robineau-Desvoidy (Szpila, 2004). Within these morphologically very similar genera, *O. austriaca* Villeneuve can be eliminated, as this species has multicuspoid spines on abdominal segments I–VII, and *B. viarum* (Robineau-Desvoidy) possesses tiny spines lateroventrally on abdominal segments I–VII (Szpila, 2004). *Bellardia vulgaris* (Robineau-Desvoidy) and *O. floralis* both have mouthhooks and cuticular spines matching the original figures for *A. chrysalis* (compare Fig. 1 and Fig. 2C, D), but, whereas *B. vulgaris* has the anal division equipped with large papillae around the spiracular field (Fig. 2F, H), the same papillae of *O. floralis* are almost invisible (Fig. 2E, G). Further diagnostic information comes from the presence of a row of spines ventrally on the anal division of *A. chrysalis* just posterior to the complete circle of spines (Fig. 1; note that Mayer's figure of the first instar unmistakably is from the ventral side). This ventral row of spines is known among calyptrate flies only from species of the genus *Onesia* (Fig. 1E; Szpila, 2004, fig. 42 and unpublished). In conclusion, *Onesia floralis* presents the best fit to Mayer's figures of *A. chrysalis*, to the extent that we do not hesitate proposing a synonymy between these nominal taxa.

Nomenclature

The nomenclatural implications of this identification are summarized as follows.

Onesia

Onesia Robineau-Desvoidy, 1830: 365. Type species: *O. floralis* Robineau-Desvoidy, 1830, by designation of Townsend (1916: 8).

Acanthosoma Mayer, 1844a: 73 (also 1844b: 409). Type species: *A. chrysalis* Mayer, 1844, by monotypy. [Junior homonym of *Acanthosoma* Curtis, 1824 (Hemiptera), *Acanthosoma* Ross, 1835 (Crustacea) and *Acanthosoma* De Kay, 1842 (Pisces).] **Syn.n.**

Acanthosomella Strand, 1928: 47. New replacement name for *Acanthosoma* Mayer, 1844.

floralis

floralis Robineau-Desvoidy, 1830: 366 (*Onesia*). Type locality not given, probably France.

chrysalis Mayer, 1844a: 73 (also 1844b: 409) (*Acanthosoma*). Probably Germany, near Bonn [by inference from the address of the author]. **Syn.n.**

We here formally designate the syntype illustrated by Mayer (1844b, our Fig. 1A) as lectotype to fix and ensure the consistent interpretation of this name.

Biology

Edible frogs of the *Rana esculenta* complex are common in many places of central Europe, and they have been the focus of extensive biological and parasitological research (e.g. Smyth & Smyth, 1980). *Onesia floralis* is a widespread and common European blow fly (Rognes, 1991), and, although biological information about it is sparse, the evidence points to its being an obligate earthworm parasite or predator. Like all species of *Bellardia* and *Onesia*, *O. floralis* is ovo-larviparous (Rognes, 1991), and, even if the larval activity in the host may be expected to immobilise or even kill the host in a few days, the high abundance of *O. floralis* in many localities in central Europe from May to August (K. Szpila, personal observation) leads to the assumption that live, infested earthworms are common in at least part of the season. Therefore, it is not unrealistic to imagine recently infested earthworms being eaten by frogs, which will bring first instar larvae of *O. floralis* into the frog stomach. In the acidic and near anoxic frog stomach, fly larvae would certainly show escape reactions. Taking this entirely hypothetical scenario a step further, one could imagine first instar larvae of *O. floralis* trying to escape the hostile environment by migrating through the stomach wall, and then succumbing to the host immune system when reaching the outer stomach wall, ending up as encysted inclusions. Accepting the plausibility of such a scenario immediately raises the question why such encysted first instar larvae of *O. floralis* or other earthworm-parasitizing blow flies have not been documented more often in the literature. The high incidence of infestation in the frogs examined by Mayer (three of five dissected frogs) and the high number of fly larvae (four to ten) per infested frog would seem to indicate that the phenomenon should not be difficult to discover, given an observant student examining the outside of the stomach wall of dissected edible frogs. With each cyst being about 3 mm long (Mayer gave the length of his *A. chrysalis* as '1½ Linien', which equals 3.18 mm), they would be difficult to ignore, but, surprisingly, no other observations of blow fly first instar larvae in frog stomachs have been found in the literature. Mayer's observations may herewith have been settled taxonomically, but they are still asking for a more detailed biological explanation.

It should be noted that encysted first instar blow fly larvae are not entirely restricted to the observations by Mayer, as Keilin (1915) provided a figure of a first instar *Pollenia* sp. encysted in an earthworm (his Planche III, figs 8–10, 'kyste phagocytaire').

Acknowledgements

We are grateful to Dr K. Rognes, University of Stavanger, Stavanger, Dr N.E. Woodley and Dr A.L. Norrbom, USDA Washington, DC, and Dr O. Lonsdale, Smithsonian Institution, Washington, DC, for critically reading and commenting on the manuscript. KS acknowledges financial support through grant no. 2 P04F 005 29 from the Polish Ministry of Science and Higher Education. The order of authors is alphabetical: all authors contributed equally to this work.

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Accepted 20 December 2007
First published online 14 March 2008

