

## SYSTEMATICS AND PHYLOGENY

# The Strepsiptera-Odyssey: the history of the systematic placement of an enigmatic parasitic insect order

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## Abstract

The history of the phylogenetic placement of the parasitic insect order Strepsiptera is outlined. The first species was described in 1793 by P. Rossi and assigned to the hymenopteran family Ichneumonidae. A position close to the cucujiform beetle family Rhipiphoridae was suggested by several earlier authors. Others proposed a close relationship with Diptera or even a group *Pupariata* including Diptera, Strepsiptera and Coccoidea. A subordinate placement within the polyphagan series Cucujiformia close to the wood-associated Lymexylidae was favored by the coleopterist R.A. Crowson. W. Hennig considered a sistergroup relationship with Coleoptera as the most likely hypothesis but emphasized the uncertainty. Cladistic analyses of morphological data sets yielded very different placements, alternatively as sistergroup of Coleoptera, Antliophora, or all other holometabolan orders. Results based on ribosomal genes suggested a sistergroup relationship with Diptera (Halteria concept). A clade Coleopterida (Strepsiptera and Coleoptera) was supported in two studies based on different combinations of protein coding nuclear genes. Analyses of data sets comprising seven or nine genes (7 single copy nuclear genes), respectively, yielded either a subordinate placement within Coleoptera or a sistergroup relationship with

Neuropterida. Several early hypotheses based on a typological approach – affinities with Diptera, Coleoptera, a coleopteran subgroup, or Neuropterida – were revived using either a Hennigian approach or formal analyses of morphological characters or different molecular data sets. A phylogenomic approach finally supported a sistergroup relationship with monophyletic Coleoptera.

«*Systemata entomologica perturbare videtur cum ex ordinibus omnibus repellatur – animalculum – animus excrucians. Tempus ducamus, et dies alteri lucem afferent.*»

[We see the entomological system confused, and that it (the strepsipteran) bounces out from all orders. The little critter gets on our nerves. Time will show, and other days will bring light in this matter.]

Latreille, 1809

## Introduction

Strepsiptera are arguably one of the most enigmatic and fascinating groups of Hexapoda (*e.g.*, Pierce, 1909; Ulrich, 1943; Kinzelbach, 1971a; Kathirithamby, 1991, 2009; Pohl & Beutel, 2008). The highly specialized insects were addressed as *twisted parasites from outer space* (Proffitt, 2005) and the term *The Strepsiptera problem* was coined by Kristensen (1981) referring to the tremendously difficult phylogenetic placement (*e.g.*, Kinzelbach, 1990; Kristensen, 1999; Beutel & Pohl, 2006a; Trautwein *et al.*, 2012). Systematic affinities with many different groups of insects were suggested since the first species was discovered by Rossius (1793), and strong controversies continued until very recently (*e.g.*, McKenna & Farell, 2010; Ishiwata *et al.*, 2011; Niehuis *et al.*, 2012).

Strepsiptera are one of the smallest groups of holometabolan insects. Approximately 600 extant species are described and a limited number of fossils (*e.g.*, Kinzelbach & Pohl, 1994; Grimaldi *et al.*, 2005; Pohl *et al.*, 2005). One characteristic feature is the obligatory endoparasitism of larvae (all extant groups) and of the females of all but two families (Stylopodia, ca. 97% of the known species) (see Kathirithamby, 2009 for a review). This is linked with hypermetamorphosis, with extremely small first instar larvae (Pohl, 2000), and with a strong structural simplification of the secondary larval stages (*e.g.*, Pohl & Beutel, 2008). The specific type of parasitism has apparently also resulted in far-reaching morphological modifications in the short-lived adults males, and to an even higher degree in the females, especially the endoparasitic ones (Stylopodia).

Strepsiptera have always attracted the attention of entomologists (see *e.g.*, Kinzelbach, 1978; Kathirithamby, 1991). Milestones were studies by Ulrich (*e.g.*, 1927a,b 1943, 1956, 1966) and Kinzelbach (*e.g.*,

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Key words: Strepsiptera, phylogeny, placement, history.

Contributions: the authors contributed equally.

Acknowledgements: support for investigations related to this study came from the German Science Foundation (DFG, BE 1789/8-1). This is gratefully acknowledged.

Received for publication: 29 April 2013.  
Accepted for publication: 4 July 2013.

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Entomologia 2013; 1:e4  
doi:10.4081/entomologia.2013.e4

1967, 1971a,b, 1978, 1990). In the last decades the investigation of the group accelerated, with an impressive number of studies on the larval morphology (e.g., Pohl, 2000; Beutel *et al.*, 2005; Osswald *et al.*, 2009), the morphology of adults (e.g., Beutel & Pohl, 2006; Beutel *et al.*, 2005; Pohl & Beutel, 2004, 2005; Hünefeld & Beutel, 2005; Koeth *et al.*, 2012), taxonomy (e.g., Kathirithamby, 1989; Pohl *et al.*, 2012), fossils (e.g., Kathirithamby & Grimaldi, 1993; Kinzelbach & Pohl, 1994; Grimaldi *et al.*, 2005; Pohl, 2009; Pohl *et al.*, 2005, 2010; Hünefeld *et al.*, 2011), host parasite associations (e.g., Kathirithamby, 2009), biochemistry (Cvačka *et al.*, 2012; Tolasch *et al.*, 2012), genetics (Johnston *et al.*, 2004; Niehuis *et al.*, 2012) and the phylogeny and evolution based on morphological characters (e.g., Pohl, 2002; Pohl & Beutel, 2005; Bravo *et al.*, 2009) and molecular data (McMahon *et al.*, 2011; Niehuis *et al.*, 2012).

The purpose of the present study is to give an overview of the history of the systematic placement of Strepsiptera and to elucidate effects of different systematic approaches.

## Materials and Methods

The information provided here was entirely extracted from the literature (e.g., Ulrich, 1943; Hennig, 1969; Kinzelbach, 1971a,b; Kathirithamby, 1989, 1991). However, the interpretations are backed up by intensive research on the phylogeny and other aspects of the group over more than a decade (e.g., Pohl, 2000; Beutel & Pohl, 2006b; Pohl & Beutel, 2005, 2008; Pohl *et al.*, 2005, 2012; Bravo *et al.*, 2009; Koeth *et al.*, 2012; Niehuis *et al.*, 2012).

## Results

### Discovery and early systematic assignments

The first strepsipteran species was discovered in the late eighteenth century by P. Rossi (Rossius, 1793). Based on the endoparasitic habits, the author assigned the species *Xenos vesparum*, a parasite of the vespid *Polistes* Linnaeus, to the hymenopteran family Ichneumonidae. Kirby (1802) described the species *Stylops melittae* parasitizing andrenid bees and later introduced the separate order Strepsiptera (Kirby, 1815) (Table 1). In contrast to Rossi (Rossius 1793) the prominent French zoologist P.A. Latreille placed *Xenos vesparum* in a dipteran group *Phthiromyae* (Latreille, 1809) but later removed it as a separate order Rhipiptera (Latreille 1817; see also Jeannel, 1951). A close association with a dipteran group, in this case *Rhipidoptera*, was hypothesized by the famous evolutionary biologist J.B. de Lamarck (Lamarck, 1816). Like strepsipterans, the megadiverse holometabolous order Diptera is characterized by halteres, wings modified as club-shaped gyroscopic sense organs (Ulrich, 1930; Pix *et al.*, 1993). However, these structures are inserted on different thoracic segments in the two groups. The more or less intuitive placement of Lamarck (1816) was later followed by Newman (1834) who studied the morphology of a species of *Stylops*, and also by Pierce (1918). Newman's study (Newman, 1834) was already heavily criticized by Westwood (1939). Nevertheless, Pierce (1936, 1952) united the strepsipterans with Diptera and Coccidina (Sternorrhyncha, Acercaria) in a group *Pupariata*, based on the puparium occurring in the postembryonic development of these lineages, the occurrence of wingless females, ovovivipary, and the presence of *balancers*. Even by that time this implied ignoring (or violating) well established (and later confirmed) systematic units such as the widely accepted Hemiptera (or Hemipteroidea) and Holometabola (e.g., Weber, 1933; Snodgrass, 1935). As it was often the case in earlier attempts to classify insects or other groups of organisms (Typologie, see e.g., Beutel *et al.*, 2009), one

character complex was arbitrarily selected as a criterion for unifying groups, in this case the formation of a puparium.

Burmeister (1837) in his *Handbuch der Naturgeschichte* suggested a placement close to the parasitic beetle family Ripiphoridae for the first time. Both groups share endoparasitic habits, a hypometamorphic postembryonic development, and very small and quite similar primary larvae (*triungulins*) (Figure 1). Moreover adults of some ripiphorid species are strikingly similar to strepsipteran males, with flabellate antennae, strongly reduced mouthparts, and distinctly reduced elytra almost resembling halteres (Figure 2). The hypothesis suggested by Burmeister (1837) was followed by several other 19<sup>th</sup> century researchers such as for instance the French coleopterists T. Lacordaire and J. L. LeConte (e.g., Schaum, 1865; Saunders, 1872; Lawrence & Newton, 1995).

A relationship with Coleoptera was also suggested by the palaeontologist Handlirsch (1903), however in an ambivalent way. Whereas his tree (first table presented in Handlirsch, 1903) suggests a sistergroup relationship between both orders his statement *von Coleopteren abstammen* (descendants of coleopterans) suggests a subordinate placement. Based on posteromotorism (metathorax as main or exclusive flight organ) and related features a close relationship between Strepsiptera and Coleoptera was also supported by Börner (1904) in a study on the phylogeny of Hexapoda. The same concept was implicitly used by Shipley (1904) who listed both groups as neighboring independent orders.

In a detailed discussion of thoracic characters Crampton (1931) pointed out correctly that possible affinities of Strepsiptera and Ripiphoridae are based on rather superficial similarities of the larvae,

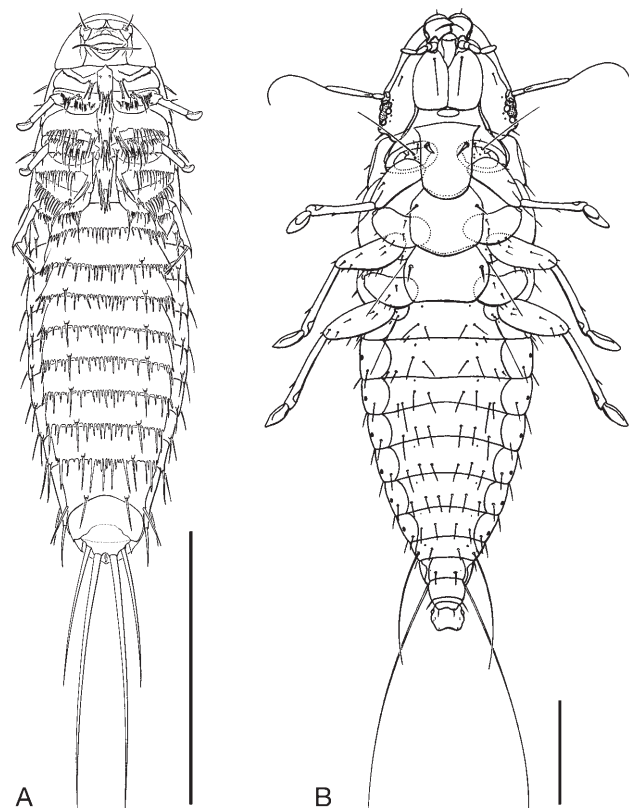


Figure 1. A. First instar larva of *Eoxenos laboulbenei* (Strepsiptera, Mengenillidae), ventral view. B. First instar larva of *Macrosiagon ferrugineum* (Coleoptera, Ripiphoridae), ventral view. Scale bars=100  $\mu$ m. (A. Modified from Pohl 2000; B. Modified from Grandi 1936).

**Table 1. Systematic placements of Strepsiptera with different approaches and data.**

Systematic approach/study	Characters	Systematic placement
<b>Pre-Hennigian concepts</b>		
Rossius (1793)	Parasitism	Hymenoptera (Ichneumonidae)
Latreille (1809)	Morphology and development	Diptera (Phthiomyae)
Latreille (1817)		Strepsiptera as separate order <i>Ripiptera</i>
Lamarck (1816)	Morphology and development	Diptera
Newman (1834)	Morphology and development	Diptera
Burmeister (1836)	Morphological similarity	Close to Ripiphoridae (Coleoptera, Cucujiformia)
Schiodte (1862/63)	Morphological similarity of larvae	Close to Ripiphoridae (Coleoptera, Cucujiformia)
Gerstaecker in Peters <i>et al.</i> (1863-1875)	Larval morphology (based on Newport)	<i>Neuroptera</i> (Neuropterida, Trichoptera, Mecoptera), closest to Phryganeidae (Trichoptera)
Pierce (1936, 1952)	Puparium	<i>Pupariata</i> , Strepsiptera united with Diptera and Coccoidea (Hemiptera)
Crampton (1931)	Metathoracic features	Common ancestor with Coleoptera
Bohart (1941)	Morphology	<i>Closely allied</i> to Coleoptera
Jeannel (1945, 1951)	Morphology, parasitism	Within Hymenoptera ( <i>apparenté aux Hyménoptères Symphytes</i> )
Crowson (1955)	Informal character discussion	Subgroup of Cucujiformia (Polyphaga, Coleoptera)
Crowson (1981)	Informal character discussion	Subgroup of Cucujiformia (Polyphaga, Coleoptera), close to Lymexylidae
<b>Hennigian concepts</b>		
Kinzelbach (1967)	Cephalic morphology	Close relationship with Mecoptera (Mecopteroid complex, Trichoptera, Zeugloptera, Lepidoptera)
Hennig (1969)	Discussion of morphological characters	Tentatively as sistergroup of Coleoptera (fig. 95)
Kinzelbach (1971a)	Discussion of morphological characters	Sistergroup of Coleoptera
Boudreaux (1979)	Mainly characters discussed by Kinzelbach (1971)	Sistergroup of Coleoptera
Kathirithamby (1991)	Posteromotorism	Sistergroup of Coleoptera
Kristensen (1999)	Discussion of morphological and developmental characters	Unresolved between Coleoptera + Neuropterida and Hymenoptera + Mecoptera, possibly sistergroup of remaining Holometabola
Willmann (2005)	Discussion of morphological characters	Sistergroup of Coleoptera
Kukalová-Peck & Lawrence (1993)	Wing base and venation	Sistergroup of Coleoptera
<b>Cladistic approach based on morphology</b>		
Whiting <i>et al.</i> (1997)	Morphological data extracted from literature	Sistergroup of Antliophora
Hörschemeyer (2002)	Wing articulation	Tentatively placed in a clade with Coleoptera and Neuropterida
Beutel & Gorb (2001)	Characters of adults and larvae including attachment structures	Sistergroup of Coleoptera (Coleopterida concept)
Kukalová-Peck & Lawrence (2004)	Wing base and wing venation	Sistergroup of Coleoptera
Beutel & Gorb (2006)	Characters of adults and larvae including attachment structures, Mantophasmatodea and some developmental features added	Sistergroup of remaining Holometabola
Friedrich & Beutel (2010)	166 thoracic characters (30 holometabolan terminals, 4 outgroups)	Sistergroup of Coleoptera (Coleopterida concept)
Beutel <i>et al.</i> (2011)	356 characters of adults and larvae (same sampling as in previous study)	Sistergroup of Coleoptera (Coleopterida concept)
<b>Single gene analyses</b>		
Chalwatzis <i>et al.</i> (1996)	18S rRNA	Sistergroup of Diptera
Whiting <i>et al.</i> (1997)	18S rRNA (alignment with MALIGN)	Sistergroup of Diptera (Halteria concept)
	28S rRNA (alignment with MALIGN)	Sistergroup of Diptera (Halteria concept)
	18S, 28S rRNA + morphological data extracted from literature (total evidence)	Sistergroup of Diptera (Halteria concept)
Wheeler <i>et al.</i> (2001)	18S rRNA (analysed with POY)	Sistergroup of <i>Metajapyx</i> (Diplura)
	28S rRNA (POY)	Sistergroup of <i>Callinectes</i> (Crustacea, Decapoda)
	18S, 28S rRNA + morphological data extracted from literature (total evidence)	Sistergroup of Diptera (Halteria concept)
Whiting (2002)	18S rRNA (182 taxa, POY)	Sistergroup of Diptera (Halteria concept)
Hayward <i>et al.</i> (2005)	USP/RXR (ecdysone receptor, Ultraspiracle proteins)	Not with Diptera
Wiegmann <i>et al.</i> (2009)	6 single copy nuclear genes	Sistergroup of Coleoptera (Coleopterida concept)
McKenna & Farrell (2010)	7 single copy nuclear genes + 18S and 28S rRNA 7 single copy nuclear genes	Polyphaga (subordinate in Coleoptera) Sistergroup of Neuropterida
Ishiwata (2011)	Catalytic subunit of DNA polymerase delta, 2 large subunits of RNA polymerase II	Sistergroup of Coleoptera (Coleopterida concept)
<b>Genomic approach</b>		
Cameron <i>et al.</i> (2009)	Mitochondrial genomes	Strepsiptera included but not mentioned
Niehuis <i>et al.</i> (2012)	Entire genomes (Neuropterida not included)	Sistergroup of Coleoptera (Coleopterida concept) (Neuropterida + Strepsiptera not completely excluded)

obviously linked to a similar type of parasitism, and that adult features rather support a common ancestry of the two orders than a subordinate placement of Strepsiptera.

A classification appearing unorthodox from today's perspective was presented by K.E.A. Gerstaecker in Peters *et al.* (1863-1875: *Handbuch der Zoologie*, Vol. II), with a large *ordo* Neuroptera, which did not only included the three neuropterid orders but also a family *Panorpina* (*Panorpa*), and Strepsiptera and Trichoptera, both classified as a *Zunft*. He considered strepsipterans as most closely related to the trichopteran family Phryganeidae, based on the alleged presence of sac-like branchiform organs. These structures were erroneously ascribed to strepsipteran larvae by Newport (1851) as already pointed out by Schaum (1865) (see also Saunders, 1872).

### Later pre-Hennigian concepts

In a detailed treatment of Strepsiptera with a focus on North American species, Bohart (1941) critically discusses earlier phyloge-

netic hypothesis. He dismisses Pierce's arguments for the *Pupariata*-concept (Pierce, 1936) (see above), and also points out that many characters shared by Strepsiptera and Ripiphoridae *are not in themselves of fundamental importance*. He emphasizes that the presence of isomerous tarsi in Strepsiptera excludes a placement among the heteromerous groups of beetles (Tenebrionoidea) as for instance Ripiphoridae or Meloidae, and concludes that the order *is closely allied to the Coleoptera*.

In the mid-20<sup>th</sup> century a variation of Rossi's Hymenoptera concept resurfaced in the famous *Traité de Zoologie* (Jeannel, 1951) and a related original study (Jeannel, 1945). The prominent French entomologist and director of the Musée d'Histoire Naturelle (Paris) R. Jeannel strongly criticized a placement of Strepsiptera close to or even within Coleoptera (Jeannel, 1951). Like Crampton (1931) he pointed out that the similarities with Ripiphoridae are superficial, and that the *postabdominal* segments of the 1<sup>st</sup> instar larvae differ profoundly (*e.g.*, terminal appendages). He emphasized numerous struc-



Figure 2. From top to bottom: *Ripidius* sp., male (Coleoptera, Ripiphoridae), length=3.1 mm; *Sepsis fulgens* (Diptera, Sepsidae), length=3.3 mm; *Stylops ovinae*, male (Strepsiptera, Stylopidae), length=4 mm.



tural affinities of strepsipteran males with members of basal hymenopteran lineages, such as for instance the orthognathous free head (*tête libre*), the distinctly reduced prothorax, the strongly developed basistarsus, and the assumed presence of numerous (vestigial) Malpighian tubules in secondary strepsipteran larvae. Jeannel (1951) assigned ordinal rank to Strepsiptera, but implicitly suggested a subordinate placement in Hymenoptera (*...détache d'une ligne primitive...*). His concept was flawed by an insufficiently detailed knowledge of strepsipteran morphology and his systematic approach based on similarity, as in earlier typological approaches. The listed structural affinities between Strepsiptera and *symphytans* are either plesiomorphic, not part of the strepsipteran groundplan, unspecific, or based on imprecise information available by that time.

The British scholar and entomologist R. A. Crowson was an outstanding figure in the investigation of Coleoptera in the 20<sup>th</sup> century (e.g., Vickerman, 1995; Wegrzynowicz, 1995; Beutel *et al.*, 2009). Crowson considered himself as a *lifelong Darwinist* and his phylogenetic concept was rather based on his *Darwinian* ideas and intuition, than on a consequent character analysis (Beutel *et al.*, 2009).

Crowson was convinced that Strepsiptera are only a subordinate coleopteran lineage (Crowson, 1955, 1960, 1981). As features supporting affinities with Coleoptera he identified the structure of the metendosternite (metafurca), the stronger sclerotization of the abdominal sternites (compared to the tergites), the large metacoxa with a reduced motility, and the presence of a gula [at least in *Mengenillopsis* (= *Mengenilla*); Crowson, 1955]. In later studies these arguments were refuted or at least weakened. The metafurca in Coleoptera is extremely variable (e.g., Crowson, 1938, 1942; Balfour-Browne, 1961) and features shared by Strepsiptera and some representatives of Coleoptera are unspecific, the stronger sclerotization of the sternites is not a groundplan feature of Strepsiptera (Pohl *et al.*, 2005), and an identifiable gula is absent in all known adult strepsipterans (e.g., Beutel & Pohl, 2006b). The condition of the metacoxa could be synapomorphic. However, the structural affinities are also unspecific.

Crowson (1955, 1960) justified a placement within the suborder Polyphaga with the reduction of one leg segment in the 1<sup>st</sup> instar larva, and the absence of the notopleural suture in adults. He treated Strepsiptera as an independent superfamily in this megadiverse beetle suborder. However, it is evident today that the modification of the larval legs are non-homologous (e.g., Pohl, 2000) and the condition of the prothorax is profoundly different in Strepsiptera (Koeth *et al.*, 2012) and Polyphaga [e.g., Hlavac, 1973 (cryptopleura); Ge *et al.*, 2007]. The placement in the polyphagan subgroup *Symphiogastra* (Eucinetoidae, Elateriformia, Cucujiformia) (Crowson, 1955) was based on the lack of articulated larval urogomphi. This implies that these structures were present in strepsipteran ancestors, which appears unlikely considering the presence of other terminal abdominal appendages (Pohl, 2000). Moreover, a reduction of movable urogomphi has obviously taken place several times in Coleoptera (e.g., Myxophaga, Trachypachidae) (Beutel & Haas, 2000). Finally, the lack of abdominal spiracles VIII was used as an argument for an assignment to the cucujiform series. In contrast to this placement it was already emphasized by Hennig (1969) that Strepsiptera lack cryptonephric Malpighian tubules, an autapomorphy of Cucujiformia (e.g., Beutel & Haas, 2000).

Crowson (1955) pointed out affinities of strepsipteran primary larvae with those of the tenebrionoid families Meloidae and Ripiphoridae. Both coleopteran groups are characterized by hypermetamorphosis like Strepsiptera and larvae of Ripiphoridae are endoparasitic. The striking similarity with some adult ripiphorids (e.g., flabellate antennae) was also recognized by Crowson (1955). Nevertheless, he correctly pointed out that a close relationship with these lineages is unlikely as strepsipteran males have a tarsal formula of 5-5-5 (groundplan) whereas Tenebrionoidea are characterized by a formula 5-5-4 (Heteromera).

In his comprehensive work on *The Biology of Coleoptera* Crowson (1981) suggested a close relationship with the highly specialized wood-associated cucujiform family Lymexylidae. He combined both groups in a cucujiform superfamily Lymexyloidea. The lymexylid genus *Atractocerus* was already compared with Strepsiptera earlier (see e.g., Schaum 1865) due to its highly aberrant morphology (King, 1955; Pix *et al.*, 1993; functional halteres). However, it is evident that many features displayed by this genus are absent in the lymexylid groundplan.

Ross (1965) also supported a status as a subordinate coleopteran group and a placement in the series Cucujiformia. He pointed out that the reduced wings, the *triungulin larva*, the reduced wing venation, and the parasitic life style suggest a close relationship with the tenebrionoid family Meloidae. However, the similarities in the morphology and development are superficial at best. Strepsipteran primary larvae differ strongly from all meloid larvae (e.g., Ge *et al.*, 2012) and aside from this it is inappropriate to refer to them as *triungulins* (e.g., Pohl, 2000).

In his last study published more than five decades after his first contribution on Strepsiptera Pierce (1964) vehemently argued against a close relationship with Coleoptera, especially a subordinate placement in Cucujiformia. This reaction was apparently inspired by the inclusion of Strepsiptera in Coleoptera in R. Arnett's *Beetles of the United States* (Arnett, 1963). While most of his arguments referring to a subordinate polyphagan placement are conclusive, the differences between beetles and strepsipterans he listed are definitely not in conflict with a sister-group relationship between both orders.

## The Hennigian period

The German dipterist W. Hennig not only revolutionized systematic methods in the middle of the last century (Hennig, 1950, 1966) but also placed insect systematics on a sound scientific basis in his groundbreaking work *Die Stammesgeschichte der Insekten* (Hennig, 1969) (see e.g., Beutel *et al.*, 2009).

Hennig emphasized the uncertainty of the phylogenetic placement of Strepsiptera. He thoroughly discussed the possible relationships of the group, implicitly favoring a sistergroup relationship with monophyletic Coleoptera (Hennig, 1969: fig. 95).

An outstanding figure in the investigation of Strepsiptera was R. Kinzelbach, who tremendously increased the morphological knowledge of the group (e.g., Kinzelbach, 1967, 1971a; Kinzelbach & Pohl, 1994; Pohl *et al.*, 2005). In his PhD thesis on adult strepsipteran head structures Kinzelbach (1967) tentatively considered a close relationship with the Mecoptera (Mecopteroidkomplex), especially with Trichoptera and Lepidoptera (*Zeuglopteren und Lepidopteren*). He listed several structural affinities (e.g., broadened head capsule, strongly convex compound eyes, processus subgenales), but discarded this working hypothesis in his comprehensive study published few years later (Kinzelbach, 1971a). In this work he listed three remaining serious options, i) a subordinate placement within Coleoptera (e.g., Crowson, 1955), ii) a sistergroup relationship with monophyletic beetles (e.g., Ulrich, 1943), and iii) an early branch distantly related to Neuropterida.

An informal evaluation of characters of the wing base and wing venation was presented by Kukalová-Peck & Lawrence (1993). The authors suggested a clade Coleopterida including Strepsiptera and monophyletic Coleoptera based on six potential wing apomorphies (or *shared autapomorphic trends*). Their interpretation of the strepsipteran wing venation was criticized by Whiting & Kathirithamby (1995). However, this in turn was refuted by Kukalová-Peck & Lawrence (2004).

The wing base of Holometabola was analyzed by Hörschemeyer (2002). The author examined and discussed Strepsiptera but pointed out that no striking synapomorphies link strepsipterans with other groups of holometabolan insects.

A completely new option was discussed by the outstanding Danish entomologist N.P. Kristensen (1981, 1999), a position outside of Holometabola (or as sistergroup of all the remaining endopterygote lin-

eages). He pointed out that in contrast to the typical holometabolous development compound eyes appear before the pupal stage (in secondary larvae) in Strepsiptera. His idea was later adopted by Beutel & Pohl (2006a), who added that primary larvae of Strepsiptera possess eleven abdominal segments in contrast to all other holometabolous groups [except for Boreidae (Mecoptera); Beutel *et al.*, 2011] and that the terminal bristles are likely cerci, which are otherwise missing in holometabolous larvae. An additional argument was the presence of external larval wing buds as distinct convexities, in contrast to the typical endopterygote condition, which is usually considered as an autapomorphy of Holometabola.

### Cladistic approaches based on morphology

The first formal cladistic analysis of Holometabola was carried out by Whiting *et al.* (1997) in a study with a main focus on molecular data. The morphological characters, which were largely extracted from the literature (*e.g.*, Kristensen, 1975, 1981, 1991, 1995; Boudreaux, 1979), yielded a placement of Strepsiptera not suggested before, as the sistergroup of a clade comprising Diptera, Siphonaptera and Mecoptera (Antliophora). However, the presumptive clade Strepsiptera+Antliophora was overruled by the total evidence analysis in the same study (see below).

The first cladistic treatment of the entire Hexapoda was published by Beutel & Gorb (2001), a study mainly focused on the evolution of attachment structures, but based on a broad array of morphological features of different body regions and life stages. A sistergroup relationship between monophyletic Coleoptera and Strepsiptera was confirmed even though posteromorphism and the reduced size of the mesothorax were not included as apparently non-independent characters.

The matrix of Beutel & Gorb (2001) was modified and re-analyzed in Beutel & Gorb (2006). The order Mantophasmatodea described in 2002 (Klass *et al.*, 2002) was included and several characters of the postembryonic development. The analysis resulted in a different placement of Strepsiptera, as the sistergroup of all the remaining holometabolous orders as discussed by Kristensen (1999) and Beutel & Pohl (2006a). The monophyly of Holometabola excl. Strepsiptera was supported by the features listed above, *i.e.* the first appearance of compound eyes in the pupal stage, the complete absence of external wing buds in larvae, the absence of the larval abdominal segment XI, and the absence of larval cerci (Beutel & Gorb, 2006).

In a study based on 126 thoracic features and 34 terminal taxa (*Goodbye Halteria?*) Friedrich & Beutel (2010) analyzed the relationships of the holometabolous orders. In the two minimum length trees Strepsiptera were placed as sistergroup of Coleoptera (branch support value 8) like in Beutel & Gorb (2001), but in this case mainly supported by features linked to posteromorphism. An extensive data matrix comprising 356 characters of different life stages and body parts was analyzed by Beutel *et al.* (2011) with the same taxon sampling. The parsimony analysis (1 single tree) and Bayesian inference both unambiguously supported a clade Strepsiptera+Coleoptera, with a branch support value of 6 in the most parsimonious tree.

### Molecular approaches: single gene analyses

The use of molecular data and especially of rRNA had a strong impact on the debate on the placement of Strepsiptera. That 18S rRNA sequences support a sistergroup relationship between Strepsiptera and Diptera was published in a short note by Whiting & Wheeler (1994), but without presenting data and any details of the analyses. This gene, which played a major role in the first two decades of molecular systematics, was also analyzed in a study of Chalwatzis *et al.* (1996) (between 1809 and 3316 bp). The sampling comprised 12 holometabolous species and 7 outgroup taxa. The analyses (neighbor-joining and maximum parsimony) yielded a clade Strepsiptera+Diptera as suggested by Whiting & Wheeler (1994).

Different placements of Strepsiptera were suggested by analysis of

molecular and morphological data in Whiting *et al.* (1997). Strepsiptera were placed as sistergroup of Diptera in the 18S and 28S rRNA trees, and also in the trees based on both genes combined and in the total evidence analyses (strict consensus trees). This revived hypothesis of dipteran affinities (*e.g.*, Lamarck, 1816) became known as the Halteria concept, referring to the presence of similar halteres, drumstick-shaped gyroscopic sense organs (Ulrich, 1930; Pix *et al.*, 1993), on the mesothorax or metathorax, respectively. The proponents of a sistergroup relationship between Strepsiptera and Diptera argued that the insertion of the halteres on different segments may be due to a shift in the homeotic gene cluster (Whiting & Wheeler, 1994), but did not present specific evidence supporting such a scenario.

Interestingly the results differed in analyses based on the same genes and a similar morphological data set conducted few years later (Wheeler *et al.*, 2001). The morphological tree also showed a sistergroup relationship with Antliophora and the 18S tree a clade Halteria. However, Strepsiptera were placed as the sistergroup of the primary wingless *Metajapyx* (Diplura) or even outside of Hexapoda in the 18S and 28S trees, respectively (Wheeler *et al.*, 2001: figs 13, 14). In contrast to Whiting *et al.* (1997) the analyses were carried out with POY by Wheeler *et al.* (2001), *i.e.* simultaneous alignment and parsimony analyses (*e.g.*, Wheeler *et al.*, 1996-2003).

A rather unusual attempt to clarify the position of Strepsiptera was undertaken by Bonneton *et al.* (2006). The authors analysed a heterodimer composed of two nuclear receptor proteins involved in the regulation of ecdysteroids – ECR (NR1H1) and ultraspiracle (USP/RXR; NR2B4). They showed that both ECR and USP/RXR ligand-binding domains experienced a strongly accelerated rate of evolution in Diptera and Lepidoptera, two orders assigned to a clade Mecopterida by Hinton (1958), Hennig (1969), Kristensen (1975, 1999) and others. As this systematic unit as defined by Hinton (1958) [see also Hennig (1969) and Kristensen (1975, 1999)] does not contain Strepsiptera, this was a valid argument against a sistergroup relationship between Strepsiptera and Diptera. However, the claim in title of the study (*...that clarifies the Strepsiptera problem*) was certainly an overstatement.

Wiegmann *et al.* (2009) analyzed 6 single copy nuclear genes with a sampling comprising representatives of all holometabolous orders. The results suggested a clade Coleopterida comprising Strepsiptera and Coleoptera with moderate support, but as only polyphagan terminals were included a subordinate placement of Strepsiptera in Coleoptera remained a possible option. A data set comprising 18s and 28S rRNA and 7 single-copy nuclear protein-coding genes was analysed by McKenna & Farrell (2010). The results based on the 7 single-copy nuclear protein-coding genes supported a sistergroup relationship with Neuropterida. Interestingly the outcome was clearly different with the 9 genes included. This data set supported a concept similar to that of Crowson (1955, 1981), with Strepsiptera subordinate in Coleoptera. A close relationship with Coleoptera was also suggested by Longhorn *et al.* (2010). Using ribosomal proteins these authors rejected the Halteria hypotheses proposed by Whiting *et al.* (1997) and Wheeler *et al.* (2001).

Ishiwata *et al.* (2011) analysed the relationships within the entire Hexapoda based on three nuclear genes (catalytic subunit of DNA polymerase delta, 2 largest subunits of RNA polymerase II). Like in Wiegmann *et al.* (2009) the results supported an arrangement [(Coleoptera+Strepsiptera)+Neuropterida] and the Halteria concept was refuted by all statistical tests.

### The age of phylogenomics

In a study of Cameron *et al.* (2009) entire mitochondrial genomes were analyzed with a fairly large taxon sampling including representatives of all orders except for Zoraptera. Interestingly, in the study Strepsiptera are not mentioned and cladograms are not shown. This suggests unorthodox and implausible placements in the analyses.

The most recent approach to solve the Strepsiptera problem was conducted by Niehuis *et al.* (2012) based on entire genomes and transcriptomes. The analyses of the former data set yielded a sistergroup relationship between Strepsiptera and Coleoptera, but Neuropterida were not included and Coleoptera were only represented by the tenebrionid *Tribolium*. In an analysis based on transcriptomes also including the archostematan beetle *Priacma serrata* LeConte the monophyly of beetles was clearly supported, thus excluding a subordinate placement of Strepsiptera within Polyphaga. The option of a sistergroup relationship with Neuropterida (McKenna & Farrell, 2010) cannot be completely excluded yet. However, it was pointed out in Niehuis *et al.* (2012) that this is highly unlikely from a morphological perspective. An enforced clade Strepsiptera+Neuropterida would require more than 20 additional steps with the morphological data set of Beutel *et al.* (2011).

## Discussion

The reasons for the prolonged uncertainties of placing Strepsiptera in the system of Hexdipoda are complex, even though the extremely modified morphology and biology stand out as the main factors. Like in molecular phylogenies with taxa with a highly accelerated substitution rate (e.g., 18S rRNA of Diptera and Strepsiptera) a reliable phylogenetic placement can be greatly impeded by numerous accumulated morphological apomorphies, in this case linked with a highly specialized biology. Reductions, for instance related to the loss or modification of wings, can lead to artifacts in phylogenetic analyses (Friedrich & Beutel, 2010), and similar life styles, especially specialized types of parasitism, can result in misleading similar features in larval stages.

The early attempts to place Strepsiptera were affected by the typological approach, i.e. a subjective choice and interpretation of features considered as typical (e.g., Beutel *et al.*, 2009). This explains easily why Strepsiptera bounced between Hymenoptera (parasitism), Ripiphoridae (similarity of primary larvae, hypermetamorphosis, some features of adults of some members of Ripiphoridae), or Diptera (halteres), or were even placed in a completely artificial unit *Pupariata* (Pierce, 1936).

Crowson (1955, 1960, 1981) attempts to place Strepsiptera were flawed by the lack of a consequent phylogenetic methodology. He was familiar with Hennigian and cladistic principles and methods but remained deeply skeptical [Crowson, 1991 (*A critique of current cladistics*); see also Beutel *et al.*, 2009]. In the light of current phylogenetic approaches and results his hypothesis of a subordinate placement within Cucujiformia is untenable. His cucujiform superfamily uniting Strepsiptera with Lymexylidae is not supported by any synapomorphic feature. Moreover, it was shown in Friedrich & Beutel (2010) and Beutel *et al.* (2011) that Coleoptera and also Coleoptera excl. Archostemata (both excluding Strepsiptera!) are well supported as monophyletic lineages.

The investigation of strepsipteran relationships became more focused with the use of the clearly defined Hennigian concepts of monophyly, synapomorphy and groundplan (Hennig, 1950, 1966; see also Beutel *et al.*, 2009). The informal (non-numerical) character evaluations tended to suggest a sistergroup relationship between Strepsiptera and monophyletic Coleoptera, but uncertainties were pointed out in all studies (see e.g., Kinzelbach, 1971a; Hennig, 1969; Kristensen, 1981, 1999). Phylogenetic signals are obscured by numerous strepsipteran autapomorphies in all life stages and were apparently largely eroded in the long evolutionary history of the order. The only obvious character suggesting a possible common origin of Strepsiptera and Coleoptera was the posteromotorism with related features such as the increased size of the metathorax or the loss of mesothoracic muscles.

The intriguing scenario with Strepsiptera as non-holometabolan

group (or sistergroup of the remaining Holometabola) (Kristensen, 1981, 1999; Beutel & Pohl, 2006a) was confirmed in a cladistic analysis of Beutel & Gorb (2006) but not in later studies based on more extensive morphological or molecular data sets (e.g., Wiegmann *et al.*, 2009; McKenna & Farrell, 2010; Beutel *et al.*, 2011; Niehuis *et al.*, 2012). This underlines that analyses based on limited character sets (and taxon samplings) can lead to erroneous results. Seemingly plesiomorphic developmental features of Strepsiptera are likely due to reversals (Niehuis *et al.*, 2012).

The early molecular investigations on strepsipteran interrelationships triggered an intensive discussion on a phenomenon called long-branch attraction [Huelsenbeck, 1997 (Is the *Felsenstein zone* a fly trap)]; see also Felsenstein, 1978]. It was pointed out by Carmean & Crespi (1995) that Strepsiptera and Diptera share a strongly accelerated substitution rate in 18S rRNA which may have caused an artificial attraction of the two branches. The long-branch effect (Felsenstein zone) mainly affects parsimony analyses and it was shown by Huelsenbeck (1997) that the 18S data analysed by Carmean & Crespi (1995) support a clade Strepsiptera+Coleoptera when maximum likelihood (ML) was used instead of maximum parsimony (MP). Huelsenbeck (1998) re-evaluated the data of Whiting *et al.* (1997) (1.0 KB of 18S rRNA for 85 terminal taxa, 0.4 KB of 28S rRNA for 51 terminal taxa) with different analytical methods (MP, ML, neighbour-joining, Monte Carlo simulation) and concluded that neither the monophyly nor the non-monophyly of Strepsiptera+Diptera is strongly supported. Similarly, Bayesian analyses of the data did not lead to conclusive results. Huelsenbeck *et al.* (2001) pointed out that the placement of Strepsiptera remains uncertain and that more sequence data from other genes are required.

The Halteria concept was already questioned by Rokas *et al.* (1999) based on a different approach. These authors showed that an intron insertion in the engrailed gene of the homeobox cluster is missing in a strepsipteran species (*Stichotrema dallatorreanum*) but is present in representatives of Mecoptera belonging to the orders Diptera and Lepidoptera.

Wheeler *et al.* (2001) is an interesting case study and played an important role in the investigation of hexapod phylogenetics. All three analyzed data sets yielded different results. It was pointed out in earlier studies that the literature-based morphological character set contained some problematic codings and the sistergroup relationship with Antliophora obviously did not reflect the true position of Strepsiptera. The 28S tree was totally in conflict with all older and current concepts, with many orders paraphyletic and a very unrealistic placement of Strepsiptera outside of Hexapoda, whereas the 18S tree finally yielded a clade *Metajapyx* (Diplura) + Strepsiptera. To construct a phylogenetic hypothesis (summary tree on p. of Wheeler *et al.*, 2001) using a combination of obviously conflicting and problematic data sets is questionable from a theoretical point of view. Moreover, the analyses with POY may have had a negative aspect. This is suggested by comparisons of the trees based on 28S rRNA in Whiting *et al.* (1997) with Strepsiptera placed in Holometabola, versus a highly unorthodox position outside of Pterygota in Wheeler *et al.* (2001). POY was criticized by Kjer *et al.* (2007) from a theoretical perspective and it was demonstrated that it performs less well than other approaches by Ogden & Rosenberg (2007).

The potential ambivalence of molecular data was demonstrated in McKenna & Farrell (2010) with two incompatible placements, one reviving the subordinate coleopteran placement of Crowson (1955, 1960, 1981), and the other one a sistergroup relationship with Neuropterida, partly in agreement with Gerstaecker's obsolete classification.

The most recent study based on entire genomes (and transcriptomes) strongly supports a sistergroup relationship with monophyletic Coleoptera. A sistergroup relationship with Neuropterida remains a theoretical option as members of this group were not included. However, this placement is highly unlikely from a morphological perspective (Beutel *et al.*, 2011; Niehuis *et al.*, 2012).



After more than 200 years the phylogenetic Odyssey of Strepsiptera almost came to an end. Problems were mainly caused by the extreme degree of specialization and a related accumulation of numerous autapomorphies. A progressive approximation towards a solution was made possible by improved morphological and molecular data sets and refined methods of phylogenetic reconstruction. Results from the international IKITE project, which is aiming at analyses of transcriptomes of 1000 selected hexapod species ([www.IKITE.org/](http://www.IKITE.org/)), are not available yet. However, preliminary analyses (B. Misof and K. Meusemann, pers. comm.) support a clade Coleoptera with monophyletic beetles as suggested by Hennig (1969), Kinzelbach (1971a, b), Kathirithamby (1991), Willmann (2005), Wiegmann *et al.* (2009), Beutel *et al.* (2011) and Niehuis *et al.* (2012).

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