The larval morphology of the spongefly *Sisyra nigra* (Retzius, 1783) (Neuroptera: Sisyridae)

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Abstract
The morphology of mature larvae of *Sisyra nigra* was studied and documented with a broad spectrum of techniques. Special emphasis is on the cephalic anatomy and on the digestive tract. Cephalic structures are highly modified, with numerous autapomorphic conditions, including a globular head capsule, an extended area with large cornea lenses, a massive tentorium, a strongly developed prepharyngeal pumping apparatus with a horizontal arrangement of dilators, a sharp bend between the prepharynx and pharynx, and an unusual filter apparatus at the entrance of the large crop. The thoracic and abdominal muscle sets, and the legs are largely unmodified. Postcephalic apomorphies are conspicuous tergal setiferous tubercles, trifid setiferous pleural projections, single pretarsal claws, zigzag-shaped abdominal tracheal gills, and a dense vestiture of setae on the terminal abdominal segments. Mandibulo-maxillary stylets curved outwards are an unusual apomorphy also found in the semiaquatic larvae of Osmylidae. Semiaquatic or aquatic habits and secondarily multisegmented antennae are potential synapomorphies of these two groups and Nevrorthidae (Osmyloidea). A sistergroup relationship between Sisyridae and Nevrorthidae suggests that fully aquatic habits of larvae may be a synapomorphy of both families. A specialized terminal antennal seta is a potential groundplan apomorphy of Neuroptera, with secondary loss in Nevrorthidae and Ithonidae + Myrmeleontiformia, respectively. A trumpet-shaped empodium is likely an apomorphy of Neuroptera excluding Coniopterygidae and Osmyleioidea, and the secondary loss an apomorphy of Ithonidae on one hand, and Myrmeleontiformia excl. Psychopsidae on the other.

KEYWORDS
larval anatomy, phylogeny, sponges, Spongillaflies

1 | INTRODUCTION

Spongeflies (or Spongillaflies) are an unusual group of Neuroptera, with fully aquatic larvae with a unique association with freshwater sponges or bryozoans (e.g., Aspöck, Aspöck, & Hölzel, 1980; Weissmair, 1994, 1999). The family was introduced by Handlirsch (1906) in his ground breaking work on fossil insects, even though Banks (1908) still included *Sisyra* Burmeister, 1839 in Hemerobiidae. Nakahara (1914) separated a tribe Neurothorini from Sisyriini, the former now recognized as a separate family Nevrorthidae (Zwick, 1967; see also MacLeod, 1964 and Aspöck & Aspöck, 2007).

The oldest record for Sisyridae is from the Cretaceous (Makarkin & Perkovsky, 2016; Perkovsky & Makarkin, 2015). Today the family comprises about 70 described species worldwide, placed in the four genera...
Sisyra, Sisyrina Banks, 1939, Sisyborina Montserrat, 1982 (Sisyridae), and Climiaca McLachlan, 1869 (Climaciinae) (Cover & Resh, 2007; Montserrat, 1982; Perkovsky & Makarkin, 2015). Seven extant species occur in Europa, five in Germany (Hölzel & Weißmair, 2002; Sauere, 2003; Weißmair, 1994, 2010), and three in Thuringia (Bellstedt & Brettfeld, 2001; Gruppe, 2015).

The adults are small and their coloration mostly dark except for the pale or mottled wings. The moniliform antennae are about half as long as the forewings, which measure between 4.5 and 7 mm (Wachmann & Sauere, 1997). Diagnostic features of adults are the reduced number of cross veins on the wings, except the costal field and genitalic features (Aspöck et al., 1980). The larvae use species of Ephydatia Lamouroux, 1816 and Spongilla Lamarck, 1816 (Porifera, Spongillidae) or colonies of Cristatella Cuvier, 1798 and Hyalinella Jullien, 1885 (Bryozoa, Plumatellidae) as hosts in stagnant and running waters (Penney & Ragel, 1968; Poirier, 1969). The postembryonic development comprises three larval stages and the pupa (e.g., Weißmair, 1999). The first instar is free-swimming over a period of 7 days, with gas exchange via the body surface. Pupation takes place in a cocoon outside of the water. After hatching and mating the females deposit about 50–60 eggs at overhanging branches near the water line (Weißmair, 1999).

The family was placed in an unresolved polytomy within monophyletic Hemerobiiformia in studies based on larval morphology (Aspöck, Plant, & Nemeschkal, 2001; Beutel, Friedrich, & Aspöck, 2010; Jandausch, Beutel, Pohl, Aspöck, & Winterton, 2018), but as sister to all the remaining Neuroptera in a contribution mainly focused on the adult head (Randolf, Zimmermann, & Aspöck, 2013). Based on anchored hybrid enrichment data (AHE), Winterton et al. (2017) placed Sisyridae in a clade with Nevrorthidae and Osmylidae (Osmyloidea). Analyses of mitochondrial genomes (Wang et al., 2017) yielded a sistergroup relationship between Sisyridae and Nevrorthidae, and a placement of Osmylidae as sister to all following families (Neuroptera excl. Coniotyridae). The observed structural features are compared to the conditions found in other groups of Neuroptera. New data and characters are added to larval character state matrices of Beutel et al. (2010) and Jandausch, Beutel, Pohl, Aspöck, et al. (2018). The extended data sets are analyzed cladistically. However, the main focus is on mapping the characters on a phylogeny based on analyses of transcriptomes (Misof et al., unpublished). Larval character evolution as implied by the phylogenetic pattern and evolutionary scenarios are discussed. This includes also autapomorphies of Sisyridae, as the main focus of this study is not on the phylogenetic reconstruction of Neuroptera, but on the larval morphology and adaptations of this taxon.

2 | MATERIALS AND METHODS

2.1 | Material

The larvae of S. nigra used for our morphological investigations were fixed in 75% ethanol. They were collected in two rivers in Thuringia (Germany), the Saale at Rudolstadt (Ordnance survey map/quadrant: 5334/1, leg Bellstedt, 29.09.2008) and the Gera close to Walschleben north of Erfurt (Ordnance survey map/quadrant: 4931/2, leg. Bellstedt am 15.09.2018).

Investigations of the macrozoobenthos of these rivers revealed freshwater sponges (Spongillidae), growing on the lower surface of large boulders. Spongely larvae were later collected in the lab from these samples using a reflected light microscope.

The ground of both rivers mainly consists of boulders of medium size. The Saale is about 40 m wide below the biodiesel factory in Rudolstadt-Scharwa, the first collecting spot. The drainage of the Saale lies in the Thuringian Slate Mountains (Thüringer Schiefergebirge) (river sequence Elbe, Weser). The source streams of the river Gera have their origin in the Thuringian forest (Thüringer Wald) (river sequence Unstrut, Saale, Elbe, Weser). At the collecting site at the Gera shortly after Walschleben, the width of the river is about 20 m and the riparian zones are partly muddy. A moderate wastewater pollution and slight turbidity was observed at both localities. Nevertheless, the limnofauna at both sites was rich. The banks of both straightened rivers were loosely lined with different trees, mostly willows in the case of the Gera.

2.2 | Scanning electron microscopy

SEM-micrographs were taken with a Philips ESEM XL30 (Philips, Amsterdam, Netherlands). One larva was dehydrated and dried with HMDS (Hexamethyldisilazan), sputter coated with gold with an Emitech K500 (Sample preparation division, Quorum Technologies Ltd., Ashford, England), and then glued to a micro-needle and fixed on a rotatable specimen holder (Pohl, 2010).

2.3 | Photomicrography

To document the coloration photos were taken with a Canon Eos 6D digital SLR equipped with a Canon MP-E 65 mm macro lens (Canon, Krefeld, Germany), fitted with a StackShot macrorail (Cogisys, Traverse City, MI). The specimens were illuminated with two flashlights (Yongnuo Photographic Equipment, Shenzhen, China). Zerene Stacker 1.04 (Zerene Systems LLC, Richland) was used to combine stacks of images with different focus. Photographs were taken in ethanol.
2.4 | Histological sections

Two specimens were embedded in Araldite CY 212 (Agar Scientific, Stansted/Essex, England). Serial cross sections were made with a Microm HM 360 (Microm, Walldorf, Germany) equipped with a diamond knife. The sections were stained with toluidine blue and pyronin G. The slides were digitalized with an Olympus dot. Slide microscope (BX51, software version 3.4, Olympus, Tokyo, Japan).

The images were arranged, and elastically aligned with ImageJ Fiji (Schindelin et al., 2012) and the Plugin Track_EM2 (Schindelin et al., 2012). The arrangement was corrected manually in Amira 6.0.1. and 6.1. (Visage Imaging GmbH, Berlin, Germany).

2.5 | 3D-reconstruction

To prepare the specimen for CT-scanning the larva was dehydrated with ethanol and then dried with CO₂ at the critical point with an Emitech K850 Critical Point Dryer (Sample preparation division, Quorum Technologies Ltd., Ashford, England). It was scanned in a SkyScan221 micro-CT (FSU Jena) with beam strength of 40 kV and 320 µA. In a 360° Scan pictures were taken e0.2° with an exposure time of 150 ms. A pixel size of 0.4 µm was achieved. Amira 6.0.1, Dragonfly software, Version 4.0 for Windows (Object Research Systems [ORS] Inc, Montreal, Canada, 2019) and 6.1. (Visage Imaging GmbH, Berlin, Germany) were used for the segmentation of the µCT-data. VGStudiomax v 2.0.5 (Volume Graphics, Heidelberg, Germany) was used for visualization and rendering (Phong).

2.6 | Image processing

All images were processed with Adobe Photoshop CS2 (brightness, tone, picture arrangement). They were also used as template for vector graphics carried out with Adobe Illustrator CS2.

2.7 | Nomenclature

The terminology for skeletal structures follows Beutel et al. (2010) and Jandausch, Beutel, Pohl, Aspöck, et al. (2018), muscles are named after v. Kéler (1963).

2.8 | Phylogenetic analysis

The data of this study were entered in a matrix with Winclada (Nixon & Carpenter, 1993) (84 characters of larvae, 2 characters of adults [apomorphies of coleopteran outgroups]; mostly from Beutel et al., 2010 and Jandausch, Beutel, Pohl, Aspöck, et al., 2018). Parsimony analyses were carried out with TNT (traditional search) (Goloboff, Farris, & Nixon, 2008) and NONA (ratchet, 1,000 replicates) (Goloboff, 1995). The character evolution is traced with Mesquite (Maddison & Maddison, 2015), using an unpublished phylogeny from the 1KITE project (http://www.1kite.org/). This tree, which is based on a set of 3,983 clusters of orthologous single-copy genes (COGs), was provided by Prof. Dr. B. Misof.

3 | MORPHOLOGICAL RESULTS

3.1 | General appearance

The body of third instar larvae appears compact. Examined specimen (n = 6) were 3–5 mm long and 1–1.2 mm broad (Figures 1 and 2). The coloration is mostly white; only the frontal area of the head and few tergal regions display a light brownish pigmentation, and articulatory elements of the leg bases are dark brown. The compact rounded head appears small compared to the postcephalic body, which reaches its maximum width at the metathorax. The dorsal postcephalic surface is characterized by large setiferous tubercles. The thoracic legs are elongate and slender. The abdomen is only about 1.3 times as long as the thorax. The abdominal segments are strongly rounded laterally. Seven pairs of segmented gills are inserted ventrolaterally on segments I–VII.

FIGURE 1 Sisyra nigra (Neuroptera, Sisyridae), larva, microphotography. (a) Dorsal view, (b) ventral view, and (c) lateral view. Scale bar: 500 µm
FIGURE 2  Sisyra nigra, larva, 3D-reconstruction. (a) Dorsal view, (b) lateral view, and (c) ventral view. Abbreviations: ag, abdominal ganglion; ang, anlagen of gonads; br, brain; cgl, cephalic gland; cmt, cryptonephric Malpighian tubule; cr, crop; fg, frontal ganglion; hg, hindgut; mg, midgut; mt, Malpighian tubule; rec, rectum; sm, stemmata; soesc, suboesophageal complex; tg, thoracic ganglion. Scale bar: 250 μm
The abdomen is strongly tapering posteriorly, with segments VIII–X distinctly decreasing in size.

3.2 | Head capsule

The cuticle is largely unpigmented and thin, but distinctly sclerotized on the dorsal side and laterally (Figures 1, 3, and 8). The head appears orthognathous due to its globular shape, but, as the mandibulo-maxillary stylets are directed anteriorly, it is in fact prognathous. The head capsule is rounded anteriorly and laterally and wider than long; in lateral view it appears short and compressed in the longitudinal direction, with the distance between the dorsal and ventral surface exceeding the length from the anterior margin to the neck region. An indistinct dorsal ecdysial suture is present, with a short posterior unpaired coronal suture dividing the dorsal occipital region. The ocular area with six large stemmata is not elevated, but comprises a large part of the lateral cephalic region. The large elliptical antennal insertion areas are located anteromesad the ocular area, almost at the anterior edge of the head capsule; a ring-shaped articulatory membrane is exposed; an antennifer is not present. The clypeal region is delimited by an angular edge of the head capsule, between the dorsal mandibular joint and the large fissure-shaped anterior tentorial grooves; an internal frontoclypeal strengthening ridge is missing. A distinct subgenal ridge with an oblique posteroventral orientation is present between the mandibular base and the posterolateral margin of the head capsule; posteriorly it is continuous with the postoccipital ridge. The broad and deep posterior tentorial pits are located ventrolaterally at the membranous hind margin of the head capsule. The ventral side of the head is formed by the extensive, membranous, and completely undivided labium, and the sclerotized but slender proximal maxillary elements. The low postoccipital ridge encloses the foramen occipitale laterally and dorsally; paired posteriorly directed projections are present dorsolaterally. A membranous collar-like cervix with a pair of small cervical sclerites connects the head with the prothorax.

3.3 | Internal skeletal structures

The tentorium is strongly developed (Figures 4 and 6). The broad anterior arms are posteriorly continuous with posterior arms of similar width; together they form massive, nearly horizontal, bars connecting the frontal cephalic region with the posteroventral margin of the head capsule. Short dorsal arms arise from these bars in the middle region of the head. A massive tentorial bridge is present close to the foramen occipitale; it bears a pair of posteriorly projecting plate-like structures; an unpaired tendon arises from the bridge anteromedially.

3.4 | Stemmata

Six well-developed stemmata with large and distinctly convex corneal lenses are present and cover a large area on the lateral side of the

**FIGURE 3**  *Sisyra nigra*, larva, SEM-micrographs of the head. (a) Dorsal view, (b) ventral view, (c) lateral view, and (d) frontal view. Abbreviations: ants, antennal socket; atg, anterior tentorial groove; cd, cardo; cer, cervix; cp, clypeus; fr, frons; hc, head capsule; lb, labium; md, mandible; mxs, maxillary stylet; ped, pedicellus; scp, scapus; sgr, subgenal ridge; sm, stemma; stp, stipes. Scale bar: 100 μm
head (Figures 1–5, and 11). They are arranged in a dorsal row of three and a lateral row of three, the former slightly shifted posterad. Both rows are slightly curved.

3.5 | Labrum

The labrum appears incompletely fused to the head capsule (Figure 3). A transverse furrow is present on the frontal region of the head capsule, possibly representing a vestigial clypeolabral suture.

Musculature: Labral muscles are completely absent.

3.6 | Antenna

The strongly elongated multisegmented antenna is composed of an elongate cone-shaped basal segment, a strongly elongated second antennomere, and a distal part composed of 14 subsegments (Figures 1 and 6). The slender distal segments are parallel-sided and evenly sclerotized. The last two segments bear anteriorly pointing finger-like processes on their distal part. A specialized terminal seta (MacLeod, 1964: FITS) is inserted on the antennal apex.

Musculature: Four extrinsic muscles are present, all of them represented by a single bundle. M1/2/3/4 (M. tentoriocapalis anterior/posterior/lateralis/medialis), O: all four muscles on dorsal tentorial arms; I: on anterior, posterior, lateral, and mesal base of basal segment, respectively.

3.7 | Mandibulo-maxillary complex

The mandibles and maxillae are closely connected, forming strongly elongated and thin sucking stylets (Figures 3, 7, and 8). The mandibles are slightly larger and nearly straight. The two distinct separated proximal maxillary elements are sclerotized and slender, placed laterad the membranous labium; the slender but long distal part is connected with the mandibular stylets via a tongue and groove mechanism along its complete length. Cardo and stipes are distinctly separated and articulate by a small joint. At the base of the mandible, two sclerotized processes are present serving as attachment sites for muscles. Additionally, the mandibles bear serrations along the edge at the distal region of the stylet. Dense microtrichia are present at the tip of the maxilla, covering the ridge between both stylet elements; the function of this curtain-like structure is unclear. At the apical region, both elements of the stylets are twisted into each other and thus strongly connected.

Mandibular musculature: M11 (M. craniomandibularis internus), large muscle, divided into two subcomponents, both consisting of several bundles, O: dorsal head capsule; I: medially on small process of mandibular base with a tendon. M12 (M. craniomandibularis externus), smaller than M11, with two subcomponents, O: one unit dorsolaterally on head capsule, second ventrolaterally; I: laterally on mandibular base with a tendon. M13 (M. tentoriomandibularis) well developed and divided into three bundles, O: ventrally on anterior tentorial arms; I: laterally an internal surface of mandible.

Maxillary musculature: M15 (M. craniocardinalis), absent. M17 (M. tentoriocardinalis), strongly developed muscle composed of five nearly equal bundles with different insertion sites along the cardo, O: ventral side of tentorium, posterad M18; I: in a series from anterior to posterior on the sclerotized cardo. M18 (M. tentoriostipitalis), composed of two thin bundles, O: ventrally on tentorial bar, anterad M17; I: one bundle proximally on lateral sclerotized stipital region, second bundle slightly shifted mesad, both inserted anterior to bundles of M17. M19 (M. craniolacinialis), absent. Imms (intrinsic muscle of maxillary stylet), absent.

3.8 | Labium

The labium is represented by an extensive, largely membranous, and strongly convex area which closes the head on the ventral side (Figures 3 and 8). It is laterally bordered by the proximal maxillary parts and posteriorly by the ventral part of the membranous cervix. Anteriorly, a narrow semimembranous part of the labium projects between the bases of the paired sucking jaws. The entire structure is completely undivided. A separate prementum, palps and endite lobes are absent.
Labial musculature: a single pair of long and slender muscles with unclear homology. O: roof of anterior hypopharynx; I: ventral wall of labium.

3.9 | Prepharyngeal tube

A closed prepharynx is formed by the epipharynx and hypopharynx which are fused along their lateral edges (Figures 2, 5, and 9). A laterally open cibarium. The anterior edge of the labral region and the anterior labium are tightly fitting with each other, thus closing the functional mouth opening. Anteriorly the prepharynx divides into two separate tubes, with the median epipharyngeal and hypopharyngeal areas tightly connected. Each of the paired tubes is continuous with a sucking channel within the mandibulo-maxillary stylets. The prepharyngeal floor formed by the hypopharynx is well sclerotized and appears U-shaped in cross section. The epipharyngeal roof of the prepharynx is mostly semimembranous, but strongly sclerotized laterally ridges are present and additionally a median longitudinal apodeme-like structure which serves as muscle attachment area.

Prepharyngeal musculature: M41 (M. frontohypopharyngalis), composed of three bundles with different origin. O: first subunit on frontal region anterior to frontal ganglion laterad M44, second posterolaterad frontal ganglion, and third on posterior head capsule anterad M46; I: dorsolateral folds at posterolateral edge of prepharynx, laterad anatomical mouth opening. M48 (M. tentoriobuccalis anterior), fairly short, thick bundle, O: dorsally and ventrally on massive corpotentorium and mesally on posterior tentorial arms; I: ventral side of posterior prepharynx. M49 (M. tentoriobuccalis lateralis), short single bundle, O: anteriorly on dorsal tentorial arms; I: lateral parts of bucca, ventrad insertion of M41.

Epipharyngeal musculature: M43 (M. clypeopalatalis), large muscle consisting of five strongly developed bundles, three of similar length and diameter, and two smaller ones, with nearly horizontal orientation,
3.10 | Salivarium and salivary ducts

A salivarium and salivary ducts and associated muscles are completely absent. The hypopharynx forms a structural unit with the anterior part of the labium.

3.11 | Digestive tract and associated structures

The elongated prepharynx forms an angle of ca. 90° with the pharynx (Figures 2, 9, and 10). Accordingly, the short pharyngeal tract is steeply descending toward the prothorax. The anatomical mouth opening is marked by the position of the frontal ganglion and the insertion of M. frontohypopharyngalis (M41) and M. frontobuccalis anterior (M45). The pharynx is followed by the oesophagus, which is widening as a voluminous inguionus or crop in the posterior head region. The epithelium of the crop consists of cube-shaped cells with tree-shaped branched processes tightly connected with the intima. The intima itself is comparatively thick, with several longitudinal folds reaching into the foregut lumen. Toward the midgut, the tree-shaped processes obliterate. A proventriculus is missing and caeca are also absent. The large, sac-like midgut is indistinctly separated from the crop by a low ring-shaped fold, probably representing a vestigial valvula cardiaca. The midgut reaches from the posterior end of prothorax to abdominal segment II. It is almost as high as the thoracic and anterior abdominal segments. In longitudinal direction, it is indistinctly subdivided by two fine ring-shaped folds, with the central portions decreasing in length from anterior to posterior. The midgut cells are cylindrical and apically lined with microvilli. A peritrophic membrane could not be identified and is apparently missing. The midgut is posteriorly closed, without connection to the hindgut. Its posterior most section above abdominal ganglion I is differentiated as a knot-shaped pylorus, the site of origin of the eight Malpighian tubules. Seven of the tubules are free, each forming a loop in the abdominal lumen. In contrast, one of them is attached to the anterior part of the rectum with its distal end. It fits tightly to the wall of the rectum and is also integrated in the single layered rectal epithelium (Gaumont, 1976: tunique externe), below the intima(?), the site of origin of the eight Malpighian tubules. Seven of the tubules are free, each forming a loop in the abdominal lumen. In contrast, one of them is attached to the anterior part of the rectum with its distal end. It fits tightly to the wall of the rectum and is also integrated in the single layered rectal epithelium (Gaumont, 1976: tunique externe). The anus lies at the tip of the abdomen.

Musculature: M45 (M. frontobuccalis anterior), slender muscle, O: frontal area posterior to second bundle of M41, posterad frontal ganglion; I: dorsally on pharynx at anatomical mouth, between insertion sites of M41. M46 (M. frontobuccalis posterior), two thin bundles, O: on frons, posterad third bundle of M41; I: posterad M45 on dorsal folds, dorsad M52. M52 (M. tentoriobuccalis posterior), long and thin muscles composed of several bundles. O: posterior tentorial arms; I: ventrally posterior pharynx.

3.12 | Glands

The paired mandibulo-maxillary cephalic glands are large and elongated and directly connected to the sucking channels (Figure 4). They extend from the base of the sucking stylet to the dorsal region of the head capsule, nearly parallel to the prepharyngeal tube. The mesal and lateral glands of the maxillary stylet are missing.

3.13 | Central- and stomatogastric nervous system

The brain appears not enlarged in relation to cephalic lumen and is almost completely located within the head capsule (Figures 2, 4, and 11).
Only a small part of the posteriorly inclined protocerebrum reaches the cervical region. Distinct optic lobes are missing. An optic nerve dividing into six branches connects the brain with the stemmata. The frontal ganglion is located dorsal to the anatomical mouth opening and connected by the frontal connective to the tritocerebrum. A nervus connectivus (dorsal root of the frontal ganglion) is also present. The circumoesophageal connectives enter the suboesophageal ganglion in the ventral part of the head, the largest part of which is located in the ventral part of the cervical region and in the anteroventral region of the prothorax. Three distinct thoracic ganglia are present and located in their respective segment. The abdominal ganglionic chain is well-developed. It consists of seven paired ganglia of nearly the same size and the complex of segment VIII. Abdominal ganglia VI–VIII are more closely adjacent to each other than the anterior ones, but still distinctly separated.

3.14 | Prothorax

An unsclerotized collar-like cervix connects the head with the prothorax; it is about 2/3 as long as the prothorax along its dorsal midline and about half as wide as its posterior edge; a small cervical sclerite is present laterally; ventrally the collar is divided into two bulging transverse subunits (Figures 1, 5, and 12). The prothorax is the longest thoracic segment; it is rounded anteriorly and laterally and widens distinctly posteriorly; the posterior edges are rounded, the posterior margin slightly sinuated. A distinctly delimited tergal plate is missing, but two widely separated tergal areas with distinct pigmentation are present; they bear five large setiferous tubercles on their anterolateral region. A distinct fold separates the pronotum region from the largely membranous pleural surface. Sclerotized pleural elements are only present proximad the coxae. A short but distinct pleural suture is dorsally adjacent with a proximolateral rounded coxal condyle; it separates the posterior epimeron from the episternum and an indistinctly delimited sclerite likely representing a trochantin. A relatively small parabolic basisternum is present on the ventral side between the procoxae; it is enclosed by ventromesal episternal extensions and an additional transverse element with unclear homology. A small presternal element is inserted between these mesally narrowing structures and the posterior bulge of the ventral collar. The transverse furcasternum is unsclerotized, without recognizable sclerotized parts; invaginations indicating the position of furcal arms or a spina are not recognizable.

The forelegs are well developed, elongate, and slender. The elongate conical coxae bear a distinct knob-shaped condyle proximolaterally, adjacent with the pleural ridge. The well-developed trochanters are about 1/4 as long as the coxae. The roughly cylindrical femur is the longest element of the leg. The slender tibia is about half as long as the femur, and the slender tarsus distinctly shorter than the tibia. The single claw is elongate and slender; an empodium is not developed.
3.15 | Mesothorax

The mesothorax is slightly shorter than the prothorax (Figures 1 and 12). It appears more transverse and rectangular in dorsal view, with rounded anterolateral and posterolateral edges and slightly convex lateral margin. A distinct tergite is missing; only two widely separated relatively small brownish areas are present, each of them bearing three setiferous tubercles. The notal area is separated from the pleural region by a distinct longitudinal groove. The weakly sclerotized and unpigmented upper part of the pleuron is subdivided by a diagonal furrow, extending from the anteroventral to the posterodorsal corner; a group of three closely adjacent setiferous tubercles is present on a large anterodorsal pleural protuberance; the cone-shaped first spiracle is inserted ventrad these protruding structures. The lower pleural part comprises a triangular trochantin, and the anterior episternum and posterior epimeron, both distinctly separated by the sclerotized and dark brown pleural ridge; the ventral edges of the ventral pleural elements, which form the pleuro-coxal articulation, are also darkly pigmented. The roughly rectangular basisternum is much larger than its prothoracic equivalent; its anterolateral and posterolateral edges are slightly rounded and the lateral margins slightly convex; a short preepisternum with a rounded anterior edge is adjacent with its anterior margin; both elements are only indistinctly separated. Like its prothoracic equivalent the furcasternum is transverse and membranous; furcal or spinal invagination sites are not recognizable.

The legs are similar to the forelegs.

3.16 | Metathorax

The metathorax is similar to the mesothorax in its general configuration, but slightly shorter (Figures 1 and 12). A spiracle is not present. The episternum is larger. The large basisternum is widening anteriorly. The preepisternum is longer and broader.

The legs are similar to the fore- and middle legs.

3.17 | Abdomen

The 10-segmented abdomen is less than one third longer than the thorax; it appears compact, almost bloated in its middle region; the

FIGURE 8  *Sisyra nigra*, larva, SEM-micrographs, details of mandibulo-maxillary complex. (a) Distal part of mandibulo-maxillary complex; (b) tip of mandibulo-maxillary complex; (c) close-up of mandibular teeth; and (d) lateral view of proximal mouthparts. Abbreviations: cd, cardo; cer, cervix; cp, clypeus; lb, labium; mct, microtrichia; md, mandible; mdt, mandibular teeth; mxm, maxillary membrane; mxs, maxillary stylet; sgr, subgenal ridge; stp, stipes

FIGURE 9  *Sisyra nigra*, larva, 3D-reconstruction, cephalic digestive tract and associated musculature. (a) Lateral view; (b) dorsal view. Abbreviations: cr, crop; eph, epipharynx; hc, head capsule; hph, hypopharynx; M41, M. frontohypopharyngalis; M43, M. clypeopalatalis; M44, M. clypeobuccalis; M45, M. frontobuccalis anterior; M46, M. frontobuccalis posterior; M48, M. tentoriobuccalis anterior; M49, M. tentoriobuccalis lateralis; M52, M. tentoriopharyngalis; ph, pharynx; tt, tentorium. Scale bar: 100 μm
posterior segments are bent downwards in some specimens (Figures 1, 12, and 13). Segments I–VII appear strongly rounded laterally due to dorsolateral pleural protuberances, but the notal regions of the segments are nearly parallel-sided. Clearly delimited, sclerotized tergites are not present, but widely separated indistinctly pigmented areas are recognizable; each of these paired areas bears a transverse row of three setiferous tubercles. Segments I–IV are approximately equally broad, but the abdomen is distinctly tapering from Segment V toward the
Segments VIII–X lack pigmented areas and dorsal setiferous tubercles; their lateral edges are nearly straight and parallel-sided; segment IX is only about half as wide as Segment VIII; the terminal Segment X is small, about half the size of IX, but not pygopod-like; terminal eversible structures or hooks are lacking. The pleural regions of segments I–VIII are distinctly separated from the notal regions by longitudinal furrows; additional longitudinal furrows are present below the insertion areas of the tracheal gills. The dorsal rounded protuberances of segments I–VIII are similar to those of the meso- and metathorax; each of them bears a closely arranged group of three to five setiferous projections. The three-segmented tube-like tracheal gills are inserted ventrolaterally on segments I–VII; the curved proximal element forms a knee-like articulation with the short middle piece; the slender apical segment appears also curved in the specimens examined. The transverse sternites are completely unpigmented but lightly sclerotized and distinctly separated from each other.

Postcephalic musculature. In its general configuration the postcephalic musculature is similar to what was described for primary larvae of *Mantispa* Illiger, 1798 (Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Jandausch, Beutel, Pohl, Gorb, et al., 2018), and in the case of the prothorax also to conditions found in Chrysopidae and Coniopterygidae (Rousset, 1969, 1971).

Several bundles of dorsal and ventral longitudinal muscles are present in each of the thoracic segments, including well-developed muscles of the neck region in the prothorax. The ventral longitudinal muscles are larger, and the number of bundles is slightly increased compared to their dorsal counterparts. Several well-developed dorsoventral muscles are present in each of the three segments, most of them inserting on the

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**FIGURE 12** *Sisyra nigra*, larva, line drawings, postcephalic body. (a) Dorsal view, thorax; (b) ventral view, thorax; (c) frontal view, leg; and (d) lateral view, postcephalic body. Abbreviations: agi, abdominal gill; bs, basisternum; cer, cervix; co, coxa; cw, claw; das, dorsal abdominal sclerite; ep, epimeron; es, episternum; fe, femur; fs, furcasternum; las, lateral abdominal sclerite; ps, presternum; sp, spiracle; ta, tarsus; te, tergum; ti, tibia; tin, trochantin; tr, trochanter. Scale bar: 500 μm.
coxal base. A complex set of small pleural muscles is present, with the single bundles inserting on the coxae and on the lateral tergal and sternal regions, respectively. The prothorax differs by muscles inserting on the cervix and the posterior head capsule. Like the skeletal elements the muscle sets of the meso- and metathorax are similar. The extrinsic and intrinsic leg muscles are well-developed and similar to the pattern found in primary larvae of *Mantispa* (Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Jandausch, Beutel, Pohl, Gorb, et al., 2018).

Series of dorsal and ventral longitudinal muscles and additional groups of notopleural and pleurosternal bundles are present in the abdominal segments I–VIII. A specialized set of muscles in abdominal segments IX and X is associated with elements of the postriormost part of the digestive tract, especially the rectum.

4 | LARVAL CHARACTERS ADDED TO THOSE OF BEUTEL ET AL. (2010) AND JANDAUSCH, BEUTEL, POHL, ASPÖCK, ET AL. (2018)

1. Compactness of tentorium: (0) normally developed, with slender elements; (1) massive. The tentorium is much more massive in *Sisyra* than in other neuropteran larvae with available anatomical data (e.g., Beutel et al., 2010; Jandausch, Beutel, Pohl, Aspöck, et al., 2018; MacLeod, 1964).

2. Size of convex cornea lenses: (0) normally sized or small; (1) distinctly increased in size. Unusually large in *Sisyra*, occupying almost entire lateral head region (Figure 3c; MacLeod, 1964). Size varying in other groups but ocular area always distinctly smaller in other groups (e.g., Wundt, 1961: Figure 2; MacLeod, 1964).

3. Secondary multisegmentation of antenna: (0) absent; (1) present. Due to a secondary subdivision of flagellomeres the antennae appear multisegmented in *Sisyra*, *Nevrorthus* Costa, 1863, *Osmylus* Latreille, 1802 and *Hemerothus* Linnaeus, 1758.

4. Intrinsic muscle of the stylet: (0) absent; (1) present. Missing in *Sisyra*. Present in other groups of Neuroptera (Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Rousseau, 1966; Wundt, 1961).

5. Convexity of labial region: (0) not strongly convex; (1) strongly convex. A strongly convex labial region is a unique characteristic of larvae of *Sisyra* (Figures 3c and 8d).

6. Prementum: (0) present as defined labial subunit; (1) not present as defined labial subunit. Lacking in *Sisyra* as a recognizable structural entity.

7. Labial palp: (0) present; (1) absent. Missing in *Sisyra*.

8. Sharp bend of cephalic digestive tract: (0) absent; (1) present. A sharp bend of about 90°/C14 is present between the prepharynx and pharynx of *Sisyra*. This feature is absent in the other groups with anatomical data (e.g., Beutel et al., 2010; Gaumont, 1976; Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Wundt, 1961).

9. Arrangement of muscles of prepharyngeal sucking pump: (0) vertical or oblique arrangement; (1) horizontal arrangement. An exceptionally strongly developed prepharyngeal sucking pump is present in *Sisyra*, with strongly developed and horizontally arranged bundles of mm. 43, 44, and 48. This is a unique feature in Neuroptera as far as known at present (Gaumont, 1976: Figure 26; Beutel et al., 2010; Jandausch, Beutel, Pohl, Aspöck, et al., 2018).

10. Setiferous tubercles on postcephalic tergites: (0) absent; (1) present. Present in *Sisyra* in similar shapes and pattern in Chrysopidae (e.g., Tauber, 1987).

11. Compact pleural groups of setiferous projections: (0) absent; (1) present. Only present in *Sisyra*.

12. Claws: (0) double; (1) single; (2) absent. Single in *Sisyra* and absent in *Mantispa* (Jandausch, Beutel, Pohl, Aspöck, et al., 2018).

13. Abdominal tracheal gills: (0) absent; (1) straight; (2) zigzag-shaped. Present and zigzag-shaped in *Sisyra* (Figures 1 and 12; Weißmair & Waringer, 1994). Straight in Megaloptera.

14. Connection of midgut and hindgut: (0) present; (1) absent. Connection probably generally interrupted in larvae of Neuroptera (e.g., Gaumont, 1976).

15. Association with sweet-water sponges: (0) absent; (1) present. The association with sweet water sponges is a unique feature of *Sisyra*.

Additional characters not included in the analysis.

16. Size of ingluvies: (0) normal size; (1) enlarged. The crop of *Sisyra* appears unusually large and reaches into the head anteriorly.
However, this character is variable according to Gaumont (1976) and therefore not included in the analysis.

17. Filter apparatus of ingluvies: (0) absent; (1) formed by branched lamellae. A characteristic filter apparatus is formed by branched lamellae of the anterior crop of Sisyra. The absence if confirmed for Mantispa (Jandausch, Beutel, Pohl, Aspöck, et al., 2018) but sufficiently precise information is not available for most groups. Irregularly arranged folds are present in Nevrorthus (Beutel et al., 2010).

18. Size of midgut: (0) normal size; (1) enlarged. The midgut of Sisyra appears large, almost as high as the postcephalic body. As a precise definition of character states is not possible (e.g., Gaumont, 1976: Figure 4) this feature is not included in the analysis.

19. Rectal glands: (0) absent; (1) present. Longitudinal glands are present in the rectal ampulla of Sisyra (Figures 10 and 13; Gaumont, 1976: filière) and also in Mantispa (Jandausch, Beutel, Pohl, Aspöck, et al., 2018). Precise information for other taxa is not available.

20. Specific connection between subgenal ridge and posterior tentorial grooves: (0) absent; (1) present. A peculiar connection between the subgenal ridge was pointed out as a shared feature of Sisyra and Osmylidae by MacLeod (1964). However, as this character was not adequately described and not mentioned in the detailed study of Wundt (1961), it was not included in the analysis.

21. Dense vestiture of long setae on posterior abdominal segments IX and X: (0) absent; (1) present. The dense vestiture of long setae on the terminal abdominal segments of Sisyra is likely autapomorphic. However, a detailed documentation of this feature was not possible with the material at hand.

22. Minute teeth of the apical region of the mandibular stylets: (0) absent; (1) present. Present in Sisyra but insufficiently documented in both groups.

23. Microtrichia at the tip of maxillary stylet: (0) absent; (1) present. Densely arranged microtrichia cover the rim between the mandibular and maxillary stylets of Sisyra.

24. Stylets with twisted apical parts of mandibular and maxillary elements: (0) absent; (1) present. Present in Sisyra. Probably absent in the remaining Neuroptera (e.g., Beutel et al., 2010; Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Jandausch, Beutel, Pohl, Gorb, et al., 2018; Wundt, 1961), but not sufficiently documented in most groups.

25. Functional mouth opening: (0) open; (1) closed. Closed by a tight contact of the anterior labral edge and the anterior edge of the labium in Sisyra. A more or less complete facultative or permanent closure appears to be a general feature of Neuroptera (Gaumont, 1965: Figure 26, fermeture antérieure), with a tight interlocking mechanism in Myrmeleon Linnaeus, 1767. Presently the character is not sufficiently documented for most taxa.

26. Shape of prepharynx: (0) bottle shaped; (1) Y-shaped; (2) anterolateral branches transverse. The prepharynx is simple and bottle-shaped in Coniopterygidae and Nevrorthus, whereas it appears Y-shaped in Sisyra (Gaumont, 1976: Figure 27). The anterolateral branches of the prepharynx are transverse in...
were confirmed as monophyletic, and Psychopsidae was placed as sister to the remaining myrmeleontiform families.

6 | DISCUSSION

Larvae of Sisyridae have a highly specialized lifestyle and accordingly evolved numerous morphological apomorphies as adaptations. The close association with freshwater sponges as such is a rare if not unique feature in holometabolous insects, and correlated with an entire series of unusual apomorphies.

An ancestral aquatic lifestyle of larvae was considered as ancestral for a clade Megaloptera + Neuroptera (e.g., Aspöck & Aspöck, 2007). The recent topology of the order with the terrestrial Coniopterygidae as sister group of all remaining families (Wang et al., 2017; Winterton et al., 2017), suggests that larval development in the aquatic environment may be secondary feature. The phylogenetic pattern based on recent transcriptome analyses (B. Misof, pers. comm.; Figure 14) suggests that semiaquatic larvae may be a groundplan apomorphy of Osmyloidea, and a fully aquatic larval lifestyle a synapomorphy of Sisyridae and Nevorthidae. However, considering the striking difference in the appearance and mode of gas exchange, it appears possible or even likely that fully aquatic larvae have evolved independently in the two families. Gas exchange in the extremely slender nevorthid immatures take place via the weakly sclerotized postcephalic body surface, whereas the second and third instars of Sisyra breath via unusual zigzag-shaped abdominal tracheal gills. The specifically shaped gills, differing profoundly from those of megalopteran larvae, are apparently one of many autapomorphies of the family.

Cephalic structures of Sisyra differ strikingly from those of other neuropteran larvae, especially from the flat and elongate head of Nevorthidae (Beutel et al., 2010). The almost globular head is an autopomorphy, with an unusual anteroventral origin of the mandibulo-maxillary styloids. Another derived feature is the large group of six stigmata, occupying almost the entire lateral side of the head. The globular shape, the fairly thin cuticle, the large size of the thin cornea lenses, and the almost completely membranous ventral side reduce the mechanical stability of the head capsule. Therefore, it is not surprising that the nearly horizontal tentorium is complete and more massive than in any other group of Neuroptera (Beutel et al., 2010; Jandausch, Beutel, Pohl, Aspöck, et al., 2018; MacLeod, 1964; Wundt, 1961), obviously an additional autopomorphy of Neuroptera.

With their association with freshwater sponges (or bryozoans), larvae of Sisyridae can be considered as parasites. The specialized food uptake using immobile hosts explains further unusual features of the family. In contrast to all other neuropteran groups (e.g., Beutel et al., 2010; Gaumont, 1976; Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Wundt, 1961), larvae of Sisyra lack a poison channel, the lateral poison gland and intrinsic muscles of the stylet. Secondary reduction is likely, as feeding on immobile organism does not require injection of toxins. In contrast to the lateral gland of the stylet, the mandibulo-maxillary gland of Sisyra is unusually large, occupying a considerable portion of the lateral cephalic lumen.

Another unusual character complex is the far-reaching reduction of the labium, which is only represented by an undivided strongly convex ventral cephalic region, with the postmentum and prementum not recognizable as separate elements and the palps and palp muscles completely reduced. The typical extrinsic labial muscles are also missing. The homology of a pair of thin bundles originating from the hypopharynx remains unclear.

The cephalic digestive tract of Sisyra is also highly derived. A sharp bend between the prepharynx and pharynx is apparently autopomorph. The prepharyngeal pumping apparatus is unusually strongly developed, with a horizontal arrangement of large bundles of M. clypeopalatalis and M. tentoriocephalitis. The crop of the larva is large and reaches into the head anteriorly. An unusual feature, insufficiently documented in other groups, is a filter apparatus of the anterior crop, with characteristic branched longitudinal lamellae. The function is somewhat unclear. Considering the narrow sucking channels, it appears unlikely that coarse particles enter the digestive tract.

Little is known about the thoracic and abdominal musculature of neuropteran larvae and holometabolous immatures in general. However, the postcephalic muscular system of Sisyra appears largely unmodified, similar to the condition found in Mantispa (Jandausch, Beutel, Pohl, Aspöck, et al., 2018). Similarly, the legs do not differ distinctly from those of most other groups. Only the single claw is a derived feature and autapomorphy of the family. The absence of a trumpet-shaped epipodium (see e.g., Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Jandausch, Beutel, Pohl, Gorb, et al., 2018) is arguably a plesiomorphic feature, shared with Coniopterygidae, Osmyloidea and Nevorthidae. The presence in Psychopsidae indicates that the absence in the remaining Myrmeleontiformia is likely a secondary feature (Jandausch, Beutel, Pohl, Aspöck, et al., 2018; Jandausch, Beutel, Pohl, Gorb, et al., 2018). A derived feature shared with at least some Chrysopidae is the presence of tergal setiferous tubercles. This is likely a result of convergent evolution, as a close relationship of both families can be ruled out with reasonable certainty (e.g., Wang et al., 2017; Winterton et al., 2017). Aside from the zigzag-shaped tracheal gills, compact groups of pleural setiferous projections are an unusual apomorphy of the postcephalic body. The dense vestiture of long setae on the posterior abdominal segments is likely also autapomorph. A noteworthy feature of the rectum is the presence of well-developed longitudinal glands, designated as filière by Gaumont (1965), and also observed in first instars of Mantispa (Jandausch, Beutel, Pohl, Aspöck, et al., 2018). Even though precise anatomical observations are missing for all other groups, it is conceivable that this is a groundplan feature of the order. This raises the question whether the silk for building the cocoon is indeed produced by the Malpighian tubules (e.g., Kristensen, 1991) or in fact by the rectal glands.

The parsimony analyses of the morphological data set yielded a similar pattern as in Beutel et al. (2010) and Jandausch, Beutel, Pohl, Aspöck, et al. (2018) as expected, with the aquatic Nevorthidae as sister group of the remaining Neuroptera, monophyletic Hemerobiformia as sister group of Myrmeleontiformia, and Sisyridae as sister taxon of Osmyloidea. A distinctly different pattern is suggested by recent analyses of large molecular data sets, either based on mitochondrial genomes (Wang et al., 2017), anchored phylogenomics
(Winterton et al., 2017), or transcriptomes (3,983 COGs; B. Misof, pers. comm.). The terrestrial miniaturized Coniopterygidae are consistently placed as sister to all remaining families, Osmyloidea (= Nevrorthidae + [Sisyridae + Osmylidae]) as second branch within the order in Winterton et al. (2017), followed by Dilaridae, Mantispoidae, and Hemerobioidae, and then Ithonidae and the myrmeleontiform families (see also Engel et al., 2018). The monophyly of Osmyoidea was not supported in Wang et al. (2017), and Myrmeleontiformia, which are strongly supported morphologically (Beutel et al., 2010; Jandausch, Beutel, Pohl, Aspöck, et al., 2018), were paraphyletic in Winterton et al. (2017). Both groups were obtained as clades in the new transcriptic tree (Figure 14).

The topologies based on molecular data suggest a different pattern of character evolution as outlined in Beutel et al. (2010). In addition to the generally accepted autapomorphies of the order, such as for instance mandibulo-maxillary stylets or a posteriorly closed midgut, a specialized terminal antennal seta (MacLeod, 1964: FITS) is added as an additional derived groundplan feature, with secondary loss as an autapomorphy of Nevrorthidae on one hand and a synapomorphy of Ithonidae + Myrmeleontiformia on the other. The three osmyloid families are supported by secondarily multisegmented antennae, with parallel evolution in Hemerobiiformia. The basal position of Coniopterygidae implies that terrestrial larvae are ancestral for the order as most parsimonious interpretation. Consequently, semiaquatic or aquatic immatures are a potential groundplan apomorphy of Osmyoidea. Mandibulo-maxillary stylets curved outwards are a shared feature of Sisyridae and Osmyloidae. The placement of Nevrorthidae in the osmyloid clade implies several reversals, the secondary presence of a well-developed gula, the loss of the specialized terminal antennal seta, and a secondarily subdivided postmentum. The absence of a trumpet shaped empodium (Jandausch, Beutel, Pohl, Gorb, et al., 2018) is a symplesiomorphy of Coniopterygidae and Osmyoidea, the presence a groundplan apomorphy of the remaining families, and the secondary loss an apomorphy of Myrmeleontiformia excl. Psychopidae.

7 | CONCLUSIONS

The morphology of the highly specialized larvae of Sisyridae is mainly shaped by aquatic habits and a close association with freshwater sponges or bryozoans. Autapomorphies include modifications of the head capsule, cephalic endoskeleton, and cephalic digestive tract, a largely reduced labium, single claws, zigzag-shaped abdominal tracheal gills, and unusual pleural surface structures. The analysis of character evolution suggests that terrestrial larval development may be ancestral for Neuroptera, that a semiaquatic lifestyle is a derived groundplan feature of Osmyoidea. Distinctly different morphological adaptations suggest that fully aquatic larvae have evolved independently in Nevrorthidae and Sisyridae.

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AUTHOR CONTRIBUTIONS

R.B. collected the specimens. K.J. contributed the SEM pictures and 3D-reconstructions, and was responsible for the design of the illustrations. R.G.B supported the work in all stages and carried out the phylogenetic analysis and the line drawings. R.G.B and K.J. have written the main parts of manuscript. R.B. contributed sections on the habits and life style, and made valuable suggestions on other parts of the manuscript.

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### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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