ON THE MORPHOLOGY AND MITOCHONDRIAL DNA BARCODYING OF THE FLESH FLY SARCOPHAGA (LIOPYGIA) ARGYROSTOMA (ROBINEAU-DESVOIDY, 1830) (DIPTERA: SARCOPHAGIDAE) – AN IMPORTANT SPECIES IN FORENSIC ENTOMOLOGY

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Abstract.— Descriptions of the developmental stages of Sarcophaga (Liopygia) argyrostoma (R.-D.) are given. Scanning electron microscope images of most of its immature stages are presented for the first time. The sequence of mitochondrial cytochrome c oxidase subunit I (COI) gene fulfilling DNA barcoding standards is presented for the first time.

Key words.— Diptera, Sarcophagidae, developmental stages, forensic entomology, mitochondrial DNA, cytochrome oxidase (COI).

INTRODUCTION

Sarcophaga (Liopygia) argyrostoma is a common species in some parts of Poland; it has been recorded from the Baltic Coast, Pomeranian Lakeland, Mazovian Lowland, Lower Silesia, Małopolska Upland, Lublin Upland, Roztocze, Tatra Mts and the former Galicia (Draber-Mońko 1973, 1982, 1991, 1998).

Larvae of Sarcophaga (Liopygia) argyrostoma (Robineau-Desvoidy) are important from the point of view of forensic entomology. According to Wyss (1997), the flesh fly most often inhabits corpses kept indoors. Out of the ten records from human corpses in Switzerland, only in one case were the fly larvae found on a corpse out of doors. In Poland, in one forensic case, the fly laid larvae on a corpse in a flat (Grochowska unpublished). Knowledge of the morphology and of the duration of particular developmental stages of the fly is useful when determining the PMI (post mortem interval).

The species was originally described as Myophora argyrostoma Robineau-Desvoidy in 1830 from the Cape of Good Hope, Cape Province in South Africa. Later, it was included in various genera. Recently, the name of this species has been used in many combinations, for example: Sarcophaga argyrostoma (R.-D.) (Smith 1986), Liopygia (Thomsonia) argyrostoma (R.-D.) (Povolný and Verves 1997, Peris et al. 1999, Parchami-Araghi et al. 2001), Liopygia (= Sarcophaga) argyrostoma (R.-D.) (Grassberger and Reiter 2002, Grassberger and Frank 2004), Sarcophaga (Liopygia) argyrostoma (R.-D.) (Pape 1996), Liopygia argyrostoma (R.-D.) (Fan and Pape 1996), Parasarcophaga argyrostoma (R.-D.) (Benecke 1998, Awad et al. 2003) and Thomsonia argyrostoma (R.-D.) (Lehrer 2006).

In the catalogue of the World Sarcophagidae, it was placed in the genus Sarcophaga Meigen, 1826, the subgenus Liopygia Enderlein, 1928, with seven other species (Pape 1996: 345–348). The nomenclature used
in the present study follows the classification of Pape (1996).

Complete descriptions of the preimaginal stages of *S. (L.) argyrostroma* (R.-D.) were provided by Tölg (1913), Aldrich (1916), Thompson (1921), Greene (1925), Knipping (1936), Hafez (1940), Yates (1967), Abou-EI Ela and El-Gindi (1999) and Awad *et al.* (2003). These authors presented more or less detailed descriptions and figures of all preimaginal instars of the species. Unfortunately, they applied no modern documentation techniques. Scanning electron microscopy (SEM) is particularly useful for illustrating external characters. During recent years, several SEM-based papers appeared, dealing with larval morphology of calyptrate flies of veterinary and medical importance (Aspooas 1991, Awad *et al.* 2003, Sukontason *et al.* 2003, Pérez-Moreno *et al.* 2006 and others).

Recently, a new approach has been introduced for species identification – DNA barcoding. DNA barcoding has been proposed as a standardized approach to the characterization of life forms in numerous groups of living organisms (Hajibabaei *et al.* 2007). In animals, the selected region is the 5'-terminus of the mitochondrial cytochrome c oxidase subunit I (COI) gene (Hebert *et al.* 2003). Moreover, DNA barcoding can be used in species identification of all life stages and when only tissue fragments are available for analysis.

The main aim of this study is to provide a thorough morphological documentation of the adult and the immature stages of this flesh fly, and to estimate the COI gene sequence.

Due to its being a culturophile and a synanthrope, the species occurs in all the zoogeographical regions except Australia and New Zealand. In northern latitudes, it is closely associated with human habitations (Povolný and Verves 1997).

In this paper many SEM photographs of all the immature stages of *Sarcophaga (Liopygia) argyrostroma* (R.-D.) are presented for the first time.

**MATERIAL AND METHODS**

The larval material was obtained from females caught in the wild and kept in the laboratory. The females were collected in Warsaw, central Poland, and identified using Rohdendorf’s (1937, 1970) keys and the reference collection of the Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw. In order to obtain larvae, freshly caught females were kept individually in 750 ml glass jars with poultry liver, with finely perforated lids. For a few days, the gravid females spontaneously and repeatedly larviposited. Some of the first instar larvae were killed in hot water (ca. 95°C) to extend the pseudocephalon and to avoid subsequent deformation during storage in 75% alcohol. When offspring were numerous, some larvae were given food in an attempt to rear them to older instars, which were then killed and preserved in the same way as the first instars. Poultry liver or meat were used as food. Apart from second and third instar larvae, pupae and imagines of *S. (L.) argyrostroma* (R.-D.) were reared.

Preserved larvae were slide-mounted in pure glycerine for light microscopy. Concave slides were used for the first and second instars and the cephalo skeleton of the third instar. Preparation for SEM included dehydration in 50, 90 and 99.5% ethanol and critical-point drying in CO2. The first instar larvae and some of the second and third instars larvae were sputter-coated with platinum and SEM photos were taken by Krzysztof Szpila with the use of a HITACHI S-4700 scanning electron microscope (Figs 16–28, 55–57, 61, 62, 64–67, 83–93).

The light microscope illustrations (Figs 70–73) were produced by Krzysztof Szpila from photographs taken with the use of a digital Nikon 8400 camera mounted on a Nikon Eclipse E200 microscope.

The remaining photographs of the second and third instar larvae and pupae were coated with a gold-palladium mixture and examined in HITACHI S-3400N SEM at an accelerating voltage of 25 kV. Photographs (Figs 53, 54, 78–82, 94–120) were taken by Magdalena Kowalewska-Groszkowska and (Figs 58–60, 63, 75–77, 97, 98) by Malwina Roszkowska. The remaining photographs (Figs 1–15, 29–52, 68, 69, 74, 99, 100) were taken with a LEICA IC3D by Piotr Ślipiński.

**Terminology.** Adult terminology follows Merz and Haenni (2000), except that term bristle is used to denote a particularly strong seta. Larval terminology follows Courtney *et al.* (2000) with a few modifications proposed by Szpila and Pape (2005, 2007).

Wild adult specimens were collected in Warsaw, Poland. Flies were immediately killed and placed in 96% ethanol and frozen at −20°C until DNA extraction.

**DNA extraction.** Genomic DNA from entire thorax of three individuals was extracted using a GenElute Mammalian Genomic DNA Purification Kit (Sigma-Aldrich, Inc., Milwaukee, WI) following the manufacturer’s instructions. DNA was eluted in 100 µl H2O and their amount quantified on Spectronic Helios Beta spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA). Purified DNA was stored at -20°C in National Plant, Fungi and Animal DNA Bank in Poland www.bankdna.pl.

**Amplification and sequencing.** The Folmer region of cytochrome c oxidase 1 gene (COI) was amplified using primers described by Simon *et al.* (1994) TY-J-1460 (5'-TACAATTTTATCCTAAACTTCCAAGCC-3’) and C1-N-1840 (5'-AGGAGGATAAACAGTTCAYCC-3’) or C1-J-1751 (5’-GGATCACCTGATATAGCATTCCC-3’) and C1-N-2293 (5’-AGTAAAAACCAATTGCTAGTATAGC-3’).
PCR primers were purchased from IBB Oligo (Warsaw, Poland). Other PCR reagents were purchased from (Sigma-Aldrich, Inc., Milwaukee, WI). Each 50 µl PCR reaction mix was prepared using 25 µl 2x RedTaq Ready Mix, 4 µl of 10 mM each primer, 4 µl DNA extract, and H₂O to complete total 50 µl volume.

The thermal cycler (DNA Engine Dyad, Bio-Rad Laboratories, Inc. Hercules, CA) program consisted of an initial denaturation step of 95°C for 3 min, followed by 35 cycles of 94°C for 60 s, 45°C for 60 s and 72°C for 90 s. The final cycle was the same as the previous one except for an elongation step of 5 min duration.

Excess of dNTPs and unincorporated primers were removed from the PCR product was cleaned using Clean-Up Purification Kit (A & A Biotechnology, Gdynia, Poland). DNA was eluted in 40 µl H₂O

The sequencing reactions consisted of 30 cycles of 96°C for 20 s, 50°C for 20 s and 60°C for 4 min. Each 20 µl reaction mix was prepared using 3 µl GenomeLab DTCS Start Mix (Beckman Coulter, Fullerton, CA), 2 µl of 1.6 mM primer, 10 µl of PCR product, and H₂O to complete total 20 µl volume. All samples were sequenced with TY-J-1460, C1-N-1840, C1-J-1751, and C1-N-2293 primers in a forward direction. For sequencing products cleaning to the reaction mix were added 2 µl 3 M sodium acetate pH 4.8, 2 µl 0.1 M EDTA, 1 µl glycogen (Beckman Coulter, Fullerton, CA) and 60 µl frozen 96% ethanol. Samples were centrifuged 15 min at 12 000 g at 4°C, supernatant removed and samples dried at room temperature for 5 min. Dried samples were dissolved in 36 µl of Sample Loading Solution (Beckman Coulter, Fullerton, CA) Automated sequencing and sequence data analysis was conducted with CEQ 8000 Beckman Coulter DNA sequencer (Fullerton, CA).

Sequence data analysis was conducted with CEQ 8000 Beckman Coulter DNA sequencer (Fullerton, CA). Sequences were assembled with CAP3 program http://ph.blili.univ-lyon1.fr/cap3.php [Huang and Madan 1999]. DNA barcode was gracially depicted with FINGERPRINT program http://evol.mcmaster.ca/fingerprint/index.php [Lou and Golding 2007].

**TAXONOMY**

Genus *Sarcophaga* Meigen, 1826: 14

**Type species.** *Musca carnaria* Linnaeus, 1758, designated by Partington (1837: 697).

Subgenus *Liopygia* Enderlein, 1928: 41

**Type species.** *Musca ruficornis* Fabricius, 1794, by original designation.

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**Sarcophaga (Liopygia) argyrolostoma**

*(Robineau-Desvoidy, 1830)*


**Adults**

**Description.** Body length 7–17 mm. Male (Figs 1–6). Body densely silvery grey or yellowish-white dusted (Figs 1–2). Head silvery white (Figs 5–6). Occiput, postgena and partly gena with white hairs (Figs 1, 2, 5, 6). Gena with some black hairs on upper anterior part. Frontal vitta, antenna and palpus black. Frontal vitta velvety black with lateral margins light grey dusted. Palpus sometimes reddish brown distally. Pedicel distally and base of 1st flagellomere brownish red. 1st flagellomere light grey dusted (Fig. 5). Thorax with grey pruinescence and black or black brown longitudinal stripes (Fig. 1). Legs black with grey pruinescence, wing hyaline, with open cell R5 and basicosta light yellow white (Fig. 1). Lower calyptra white.

Abdominal pattern chequered. Segment VII+VIII black or brownish red, lustrous, with grey pollinosity, epandrium shining red (Figs 2–4).

Frons at narrowest part 0.25 head width (Figs 1, 5). Frontal vitta moderately wider frontoventrally. 1st flagellomere about twice as long as pedicel, and sometimes 2.5 times longer than pedicel (Fig. 5). Parafacial at level of antennal base 0.2–0.3 and gena 0.22–0.34 of eye height. Palpus long, distinctly widening apically. fr 8–14, medium-length. 1–2 rows of postorbital setae. vte poorly developed or indistinct, parafacial bristles weak and short, arranged in two vertical rows, the longest reach 0.4–0.5 of parafacial width. Facial ridge with numerous short, black setae at lower 0.4–0.6. ac 0+1, long and weak; scutellum with short, crossed ap and strong, long bas, subap, and discals setae. All femora and hind tibiae on ventral side with long, dense hairs; f₁ often with hairy pv, f₂ with rows of short av and pv (ctenidium), f₃ with a row of strong av; t₃ with numerous long av and pv. Costal spine small or absent, m-cu vein more or less sigmoid (Fig. 1); ratio of 3rd and 5th costal sections 1:0.5–0.8. Abdominal tergite III without medial marginal setae. Sternite V with numerous short marginal hair-like bristles and with a large “window” (Fig. 4). Segment VII+VIII moderately elongate, with several hairy marginals (Figs 2, 3). Cerci angular at dorsal subapical margin and apically spinose. Sursysty distinctly thickened along basal margin. Aedeagus with a large distiphallus (Figs 2–4).

Female. (Figs 7–15). Lighter coloured (Figs 7–10). Frons equal to or slightly narrower than eye width (Fig. 10). Frons narrowest part 1/3 of head width (Figs 7, 9, 11). Orbital plates wide. Frontal stripe only 1.25–1.5
times wider than orbital plate. Palps at base brown-yellow, somewhat distended apically (Fig. 10).

Legs. Fore femur often with pv bristles hair-like. Fore femoral organ distinct and with a few cross-stria-
tions. Mid femur with complete row of av and an apical row of short stout pv bristles. Mid-femur organ (Fig. 15) in the form of an elongate ovate patch, blackish grey coloured with diometrical lines, situated on posterior surface of mid femur. Mid femoral organ large with many cross-stria-

Abdominal tergite VI brown to reddish, grey dusted, complete, with long and short marginal bristles and hairs (Figs 7, 8, 12). Sternite VII with marginal tubercle near anterior margin and 1–2 pairs of short latero-
marginals (Figs 12, 13). Female terminalia very short, dark red or brownish red, formed by undivided tergites VI–
VIII with corresponding sternites, a pair of cerci, an epi-
proct, and a hypoproct (Figs 12, 13). Three almost uniform segmented spermathecae (receptacula seminis) pyriform (Fig. 14). S. (L.) argyrostoma is larviparus.

Ecology. The flies visit decomposing substrates – faeces and carcasses – and feed also on flowers. The larvae develop in decaying meat or prey on fly maggots in faeces (Aldrich 1916, Rohdendorf 1937, Zakharova 1961, 1965, Leclercq 1976) and on liver (personal observation). In Belgium, Leclercq (1976) reported this species from a corpse in an advanced state of putrefaction. In Great Britain (Smith 1986), Switzerland (Wyss 1997), Germany (Benecke 1998), Austria (Grassberger and Reiter 2002), Spain (Castillo 2002) and Poland (Kaczorowska and Draber-Mońko 2009) the species was reported as carrion fly; it is one of the most important species in forensic entomology.

First instar larva

The general morphology of S. (L.) argyrostoma (R.-D.) was described on several occasions (see Introduction). The present description deals only with the structures that were insufficiently described earlier. The first instar larvae of S. (L.) argyrostoma (R.-D.) are typical of Calyptratae (Figs 29–30) in having a distinct pseudocephalon, three thoracic segments (termed TI–TIII below), seven abdominal segments (AI–AVII) and an anal division (AD) carrying the posterior spiracles. Each abdominal segment (AI–AVII) has lateral creeping welts (Figs 23, 25). The abdominal segments have one transverse ventral creeping welt each (Figs 25). Body length 3.0–7.0 mm.

Pseudocephalon. Each lobe of the bilobed pseudo-
cephalon bears on its antero-dorsal surface an anten-
nal dome on a basal ring (Fig. 20) and a maxillary pal-
pus (Figs 16–18). The length of the antenna is shorter than the diameter of the maxillary palpus. The maxil-
lary palpus is located on the anterior surface of the pseudocephalic lobe and has the form of concentrically arranged rings clearly distinguished from the ambient cuticular surface (Figs 16–18). The maxillary palpus has a cluster of several sensilla in the center (Fig. 18), including three sensilla coeloconica placed on distinct sockets (SC1–3), three sensilla basiconica (SB1–3), and at least one elongated sensillum close to SC1 and SC2. In the vicinity of this cluster there are additional sensil-
la coeloconica (AS1–2). The ventral surface of the pseudocephalon bears some cirri and oral ridges that to-
gether form a facial mask (Figs 16, 17, 21). Cirri close to the functional mouth orifice are irregular (Fig. 21). Two oral ridges are situated just ventral (or posterior) to the cirri (Figs 17, 21). The oral ridges are character-
istic of saprophagous flies. The labial lobe is small and without sensilla (Fig. 21). The ventral organ is small (Figs 17, 19) and situated level with the surface of the pseudocephalon (Fig. 35), laterally to the functional mouth and above the oral ridges (Figs 17, 21). The cephaloskeleton (Fig. 36) differs greatly from that of the next instars. It is much more slender and less chitinised. The cephaloskeleton consists of paired mouth-
hooks, an unpaired intermediate sclerite and paired...
dental sclerites, parastomal bars, paired vertical plates each with ventral and dorsal cornua and dorsal bridge (Fig. 36). The mouth-hooks are equal in size and sharply pointed. Their tips are curved downwards. The dental sclerites are very small, weakly sclerotised and lying on the outer side of each mouth hook. The intermediate sclerite is rather large and more or less well sclerotised, located between and ventral to the parastomal bars (Fig. 36). The dorsal cornua are larger than the ventral ones and the vertical plate is wide. The dorsal bridge is narrow and weakly sclerotised, but complete (Fig. 36).

Thoracic segments. Only the anterior margin of all thoracic segments has complete spinose bands (Fig. 29). The anterior spinose bands of the first thoracic segment are broad, with many rows of spines, especially on the ventral and lateral surfaces (Figs 16, 17). Ventrally on TI–TIII there are paired Keilin’s organs consisting of three trichoid sensilla situated in one pit (Fig. 22).

Abdominal segments. The anterior margin of segments AI–AVI is armed with complete spinose bands (Figs 23, 26), narrowed dorsally on segment AVI (Fig. 29). The anterior bands bear several rows of spines on all abdominal segments, with the number of rows decreasing toward the posterior end of the body, especially dorsally and laterally. The posterior margin of segments AII–AVII is completely encircled with spines. The posterior bands have a single row (sometimes doubled) of spines on the lateral and ventral surfaces of AI. On segment AI some ventral and dorsal spines are present, but spines are absent from the lateral surface above the lateral creeping welt (Fig. 29) (LCV). On segment AII ventral spines and two or three dorsal rows are present; such structures are absent from the lateral surface above the lateral creeping welt. On segment AIII a lateral papilla is present (Fig. 24). Between the segments there are lateral and ventral creeping wells covered by spines (Fig. 25). A transverse crevice is present in the middle of each of segments AI–AVII (Fig. 25). In each inter-segmental region there is a complete ring of black spines which are arranged in several more or less transverse rows. The spines at the anterior border are recurved posteriorly, while those at the posterior border are recurved anteriorly. The anal division has seven pairs of papillae (Figs 27, 28). The anterior-ventral surface of the anal division presents several rows of spines (Figs 29, 31). The spiracular cavity is surrounded by a ring of hair-like spines (Figs 27, 28, 31). The posterior spiracles (PS) (Figs 31–34) are situated in a cavity and each consists of two relatively wide, nearly straight slits situated close together and more or less vertical, surrounded by a very slightly sclerotized area (Fig. 28). The anal pad is situated directly posterior and ventral to the spiracular cavity. The anal papillae are large and oval. The tip of each papilla is convex and equipped with a small sensillum (Figs 27, 28). The anal orifice (AO) is situated between the anal papillae and slightly anterior to them (Fig. 27).

Forms intermediate between LI and LII

Body length 5.0 mm, width 0.8 mm. At the cephaloskeleton of 1st instar larva there is a partly developed mouth hook of 2nd instar larva, not connected with anything yet (Fig. 37). The anterior spiracula are still undeveloped. The arrangement of the setae, the structure of the posterior spiracula and the posterior body part is like that in 1st instar larvae (Figs 29, 31).

Body length 7.0 mm, width 1.0 mm. Partly developed mouth hooks of 2nd instar larva are connected with the cephaloskeleton of 1st instar larva (Figs 38, 39). The anterior spiracula are developed (Figs 38–40). The arrangement of the setae, the structure of the posterior spiracula and the posterior body part is like that in 1st instar larva (Fig. 41).

Body length 7.0 mm, width 1.0 mm. Completely developed mouth hooks of 2nd instar larva are connected with the intermediate sclerite (Figs 42–45). The anterior spiracula are well developed (Figs 44–45). The arrangement of the setae, the structure of the posterior spiracula and the posterior body part is like that in 1st instar larva (Figs 42, 46).

Yates (1967: 435–437, Fig. 2) described this developmental stage as 2nd instar larva. He regarded the presence of four mouth hooks as a characteristic feature of 2nd instar larva of S. (L.) argyrostroma (R.-D.): “The second instar occurs from approximately 20–60 hours after deposition and is easily distinguished by the presence of 4 mouth hooks (2 pairs, Fig. 2).”

Second instar larva

Body length 7.0–13.0 mm, width ca. 1.5–2.0 mm. The integument is covered by various processes and spines (Fig. 47). In shape, 2nd instar generally resembles the 1st instar larva (Figs 29–30) but there are some differences in the following: the structure of the pseudocephalon and the cephaloskeleton, the presence of the anterior spiracula (Figs 49, 49a), the stronger and more numerous spines (Figs 61–63), the structure of the posterior spiracula (Figs 51, 52, 67) and more developed papillae on the last abdominal segment (anal division), as well as the size and shape of the anal pad (Figs 47, 64–66).

Pseudocephalon. The head of this instar is very similar to that of the third instar larva. Each of the pseudocephalic lobes is provided with an antennal dome on the basal ring and a maxillary palpus (Figs 55, 60). The diameter of the basal ring of the antennal complex is
similar to that of the maxillary palpus. The maxillary palpus is like that in the first instar. The ventral organs in the 2nd instar are similar to those seen in the 1st instar (Fig. 56). The ventral organ situated above and laterally to the functional mouth opening, appears as a globular structure with one strong spine (Fig. 57). The pseudocephalon lobes are large and located close to each other (Fig. 55). The antennae are massive. The facial mask occupies a considerable anterior part of the pseudocephalon (Fig. 55). The mouth hooks are the most anterior sclerites of the cephaloskeleton (Fig. 49). The anterior part of each mouthhook has a downward-curved pointed tip. The basal part of the mouth hook bears lateral extensions. The postero-dorsal angle and the antero-ventral angles of the mouth hook are drawn out into distinct processes. The dental sclerites below each mouth hook are weakly sclerotised. Behind the intermediate sclerite, the basal sclerite consists of paired parasomal bars, a complete dorsal bridge, vertical plates, and ventral and dorsal cornua. The parasomal bars extend far anteriorly above the intermediate sclerite. Posteriorly the dorsal cornua are deeply incised, the ventral cornua have a developed window (Fig. 49).

Thoracic segments. The anterior spiracle has a long fore chamber (Figs 48, 49, 49a, 58). Each anterior spiracle contains a single row of branches consisting of 11 or 12 oval and short branches (papillae, lobe) (Figs 49a, 58, 59). Each lobe is composed of concentrically arranged rings, covered by a flap which bears a narrow, elongate slit (Fig. 59). The anterior spiracles are similar to the corresponding structure in the third instar in their shape and number of lobes, but the fore chambers are longer than in the 3rd instar. Segments TI–TIII have spine bands only anteriorly (Figs 47, 48, 53, 54). The anterior spine bands of the first thoracic segment are broad, with many rows of spines, especially on the ventral and lateral surfaces (Figs 49, 55).

Abdominal segments. The abdominal segments are armed with both anterior and posterior spine bands (Figs 47–50, 53, 61, 62). The spines of the inter-segmental rings are more developed and more numerous. Segment AI has its posterior spine bands equipped with only few spines, the posterior spine bands of segments AI–AVII are equipped with several rows of spines. The anterior spine bands bear several rows of spines on all abdominal segments, the number of rows decreasing toward the end of the body, especially laterally and dorsally. The distinct lateral creeping welts are covered by spines between the abdominal segments (Figs 61, 62). Ventrally, each of AI–AVII is equipped with a transverse furrow. Seven pairs of papillae (P1–P7) are present on the anal division. The antero-ventral surface of the anal division presents several rows of spines. The anal pad is very big, laterally with triangular processes and anal papillae (Figs 64–66). The spiracular cavity is surrounded by a ring of black, small spines (Figs 64, 65, 67). The anal papillae are developed as spherical structures (Figs 64–67). The posterior spiracles are more developed, the peritreme is wide and the two spiracular slits are larger than in the first instar larva. Each spiracular plate bears two long, linear slits set almost vertically, surrounded by an incomplete peritreme. The inner slit is nearly straight and directed outwards, while the outer slit is slightly curved medially and dorso-ventral. The outer slit is slightly longer than the inner one (Figs 51, 52). The posterior spiracles are set in a pit deeper than in the first instar, but the fore chambers are shorter than in the first instar (Fig. 52). The posterior spiracular hairs (PSH) emanate from the margin of each slit near the middle (Fig. 67). In many places, the larval integument of this instar bears various processes and warts (Figs 61–63, 66, 67).

**Third instar larva**

Body length 12–25 mm, width ca. 2.9–6.1 mm. The colour is dirty white to yellowish (Fig. 68). The larva is the widest at the eighth body segment. The integument is covered by various processes and spines (Figs 72, 85–88), more strongly developed than in 2nd instar larvae. In shape the third instar generally resembles the second instar (Figs 47), but there are some differences in the following: the structure of the pseudocephalon and the cephaloskeleton (Figs 69, 70), the structure of the anterior spiracula (Figs 71, 84), stronger and more numerous spines (Figs 85–88), the structure of the posterior spiracula (Figs 73, 74) and more developed of papillae on the last abdominal segment (anal division), as well as size of the anal pad (Figs 89, 90, 92, 94).

Pseudocephalon. The head of this instar is very similar to that of the previous one (Figs 75–80). The two pseudocephalic lobes are markedly separated by a deep groove (Fig. 76). Each bears an antennal dome on the basal ring and a maxillary palpus (Figs 75–77, 83) which is composed of several sensilla. The maxillary palpus is like that in the first instar. Numerous cirri cover the ventral sides of both pseudocephalic lobes (Figs 75, 76, 80). The cephaloskeleton is more robust than in the second instar, but has the same general shape; it is strongly chitinised and all the sclerites are nearly black in colour (Figs 69, 70). The apical part of each mouth hook has the form of a down-curved, pointed hook. The posterior part of each hook is considerably thicker than the anterior part. The dental sclerites are located below the massive basal part of the mouth hooks, each lies on the ventral and lateral side of each hook. Behind the mouth hooks there is the intermediate sclerite, with very thick and simple-edged lateral
portions. Behind the intermediate sclerite, the basal sclerite consists of paired parastomal bars, a complete dorsal bridge, vertical plates, and ventral and dorsal cornua. The dorsal cornua are longer than the ventral ones (Figs 69, 70). The parastomal bars extend far anteriorly above the intermediate sclerite. The ventral cornua have a developed window (Fig. 70); the dorsal cornua are deeply incised posteriorly, the incision occupying more than half of their total length.

Thoracic segments. The anterior spiracle is fan-shaped (Figs 70, 71, 84, 115, 116), with a short fore chamber, consisting of 11 or 12 oval and short branches (Figs 71, 116). Segments TI–TIII are equipped with spine bands only anteriorly (Figs 68, 70, 76, 78–81). The anterior spine bands of the first thoracic segment are broader than in the previous instars, and the spines are arranged alternately in over a dozen or several dozen rows (Figs 68, 80). In living larvae, they are all black in colour, in prepared slides, most of them appear pale yellow. On the dorsal side of TI, just posterior to the spine row, a rectangular elevation is present, in older larvae to a considerable extent covered in spines (Figs 78, 79). On the dorsal side of TIII, a few small papillae are present (Fig 97).

Abdominal segments. The dorsal side of segments A1–AVII bears from a few to about a dozen papillae (Figs 68, 85, 87, 97, 98). The cuticle on most of the larva body is covered in processes of various shapes and types (Figs 86–90). Ventrally each of segments A1–AVII are equipped with transverse ventral creeping welts (Figs 81, 82). Laterally, each of segments A1–AVII have distinct creeping welts covered by rows of spines between the abdominal segments (Figs 68, 81, 82). The last abdominal segment (Figs 68, 82) differs in shape and structure from the other segments. It slightly tapers posteriorly and most of its posterior surface is a deep cavity in which the posterior spiracles lie. The edge of the cavity bears 7 pairs of conical papillae (P1–P7) (Figs 91–94), the middle pair of which is the largest. The spiracular cavity is surrounded by a ring of black, spines (Fig. 101, 106). Each band lies in an intersegmental position and appears to be composed of an upper, middle and lower narrower bands (Figs 105–107). Besides these bands, numerous transverse wrinkles, papillae, sensilla and processes are present on the puparium surface (Figs 106, 112, 113).

Puparium

Length ca. 10–15 mm. The puparium is typical of the pupae coarctatae, with a tapering posterior end and a nearly rounded anterior end (Figs 99–102). The colour of the pupa barrel immediately after pupation is pale yellowish, then it changes to bright orange and gradually darkens until finally becomes dark reddish-brown or nearly blackish in colour. The anterior spiracles of the 3rd instar larva appear as minute processes situated anteriorly (Figs 103, 104). When the pupa opens, the anterior spiracula remain outside the upper flap (Figs 114–116). On the lower flap, the place of the retraction of nearly two anterior segments of the 3rd instar larva is visible (Figs 117, 118), and the cephaloskeleton of the last instar larva remains attached to its internal wall (Fig. 119). The larval spines remain visible (Figs 103, 104, 112) and the larval tubercles on the posterior segment (Figs 105–114, 120). The larval posterior spiracles also remain visible within the spiracular cavity (Figs 109, 120). The posterior spiracles form two flat button-like structures situated at the bottom of a deep groove, corresponding to those of the larva, in the last body segment. The larval posterior spiracles may be sometimes hard to see. The small fleshy processes of this segment in the larva are very small and contracted (Figs 106, 107, 110, 111, 120).

The borders between the segments appear as continuous lines which are more distinct in the middle region of the puparium (Figs 101–103, 105–108). Along these lines there are broad transverse bands of minute black spines (Fig. 101, 106). Each band lies in an intersegmental position and appears to be composed of an upper, middle and lower narrower bands (Figs 105–107). Besides these bands, numerous transverse wrinkles, papillae, sensilla and processes are present on the puparium surface (Figs 106, 112, 113).

The sequence of mitochondrial cytochrome c oxidase subunit I (COI)

Over 700 bp of the COI mtDNA gene were obtained for three individuals with primers C1-J-1751, TY-J-1460 and C1-N-1840 and are presented below:

MIIZ-TM-3

TTTTGGAATTTATATTTTATTTTTCGGAGCTTGGCG
AGGAAATAGTTAGGAATCCTCACTAGAATTCTAATTCCAGAC
AGAACTAGTCTACCTGTCAGTATTATTTGGAAGATGATCA
AATTTATATATGTTATGCTAGCCTACTGCTTTATATTAT
AATTTTTTTATAATCATACTATACATATTTGGAAGATT
TGGAACTGACTAGCTCCATATTATACGTAGCGTTCCAGA
TATAGCTTCCTCCCTGGAATTTATATTTGAGATTTGGACT
TTTACCTCCTGCATTAACATTACTACTAGTACTGTAT

ON THE MORPHOLOGY AND MITOCHONDRIAL DNA BARCODING OF THE SARCOPHAGA (LIOPYGIA) ARGYROSTOMA

471
AGTAGAAAATGGAGCTGGAACAGGATGAACTGTTTACCC
TCCCTTTACCTCTCTAATATGCTCATGGAGGACCTCTGT
TGATCTGCATTTTTTCTCTCTCTGCTGATGAGCTTCTGT
TTCATTATTAGGGAGCTATAATTTTTATTACTACGAAT
TAATATACGACAGTCTACAGTTATCTTTTGACGAAATTT
CCTTCTATCCCTACTAGCTGTCTTTCTCTACTATATTAT
TGACTATACTGACCAAAATATTAATATCTCAATTTTGG
TACCGGAGGAGGAGGATCCAAATCCTATATCAAACACT
ATTGTGATTTTTTTGTCATCCTGAGATTATTTATAATT
ATTACC

MIIZ-TM-5

ATATTGGAACTTTATATTTTATTTTCGGAGCTGAG
CAGGAATAGTAGGGAACTTCACTAAGAATTCTAATTCGAG
CAGAAGATTAGCGGACTCTCATAGAATTCTAATTCGAG
AAATTTTATAATTGTTAGTCACCTGACTGTTTTATTTA
TAATTGTTTTTTTAGTAATACCATGAAATTTTAGATGA
TAGTGAAAGAACGGAGGACCGGAGAACTGTTTACC
CTCTTTATATCTCTATCTTACATGACAAATTTTAGATGA
CTTTTACCTCGGATATACTATCTCAGTTAAGTAGA
TTTTACCTCGGAATACACTCTCTCATTAGAATTATTTAG
TAATATATAGCTCTACTAGTTACTTTTCTTCTATTAGA
CCCTTTTTTTTGCTCTATAGCGCTTTTCTCTCTCT
TCCTTCTATCCCTACTGGTACTGCTGCAGGAAATTCTA
TATTATATATCGAGCAAAATATTATAATTTGTCT
ATCCATACGGGAGGAGGATCCAAATCCTATATCAAACACT
ATTGTGATTTTTTTGTCATCCTGAGATTATTTATAATT
ATTACC

MIIZ-TM-18

GATATTGGAACTTTATATTTTATTTTCGGAGCTGAG
CAGGAATAGTAGGGAACTTCACTAAGAATTCTAATTCGAG
CAGAAGATTAGCGGACTCTCATAGAATTCTAATTCGAG
AAATTTTATAATTGTTAGTCACCTGACTGTTTTATTTA
TAATTGTTTTTTTAGTAATACCATGAAATTTTAGATGA
TAGTGAAAGAACGGAGGACCGGAGAACTGTTTACC
CTCTTTTTTTTGCTCTATAGCGCTTTTCTCTCTCT
TCCTTCTATCCCTACTGGTACTGCTGCAGGAAATTCTA
TATTATATATCGAGCAAAATATTATAATTTGTCT
ATCCATACGGGAGGAGGATCCAAATCCTATATCAAACACT
ATTGTGATTTTTTTGTCATCCTGAGATTATTTATAATT
ATTACC

Sequenced fragments fulfill barcode standards (Hanner 2005). Low intraspecies variability of COI allows limit number to three specimens for barcode establishment. Specimens MIIZ-TM-3 and MIIZ-TM-18 show 4 nucleotide sequence differences, MIIZ-TM-5 and MIIZ-TM-18: 14, and MIIZ-TM-3 and MIIZ-TM-5: 18, accordingly. Nucleotide sequence differences between specimens varied between 0.6–2.6%. Graphical depiction of barcodes is presented in Fig. 121.

**DISCUSSION**

In our climatic zone, *S. (L.) argyrostroma* (R.-D) stays mainly near human habitations. It often visits flats, attracted by the smell of decomposing animal protein. Its larvae were found on the 9th of June 1997, on a corpse of a clothed and blanket-covered man in a flat in Lublin (forensic expertise by Dr Maria Grochowska, UMCS). Seven 3rd instars larvae of *Calliphora vicina* R.-D. and two 3rd instars larvae of *Sarcophaga* (L.) *argyrostroma* (R.-D.) were studied. The man died during a heat wave in a south-west flat. The estimated period of the development of the larvae studied was 8–9 days.

The length of the development of *S. (L.) argyrostroma* (R.-D.) in Vienna was reported by Grassberger and Reiter (2002), that in St. Petersburg – by Marchenko (1980). In Warsaw, in the dark and at a constant temperature of 23°C, the development to the pupal stage lasted 10 or 11 days, the pupal stage – from 16 to 23 days. The larvae were reared on poultry liver. In St. Petersburg, at a constant temperature of 23°C, the development from the 1st instar larva to imago lasted 22.5 days, in Vienna, at a constant temperature of 25°C, the larval development to the pupal stage lasted 8 days, and of 20°C – 12.3 days. The data on larval development at the same and at different temperatures vary. The reasons may be due to various factors, such as the geographical position, the season, the lighting regime, or the kind and availability of food.

Sequence of MIIZ-TM-18 specimen has 100% identity to the previously reported sequence AF259512 from a single specimen (Wells et al. 2001), MIIZ-TM-3 99% and MII-TM-5 98%, accordingly. Short fragment from distal part of COI (GenBank AY315643) was sequenced by Zehner et al. (2004), but it is located outside standard barcode region and sequence deposited in AF259512 file. The consensus sequence derived from three specimens in this study fulfills the DNA barcoding standards and can be used for species identification by molecular methods.

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Figures 1–6. *Sarcophaga* (*Liopygia*) *argyrostoma* (R.-D.), male (photo by P. Ślipiński). (1) Dorsal view; (2) lateral view; (3) terminalia, left lateral view; (4) terminalia, ventral view, s- male sternite V; (5) head, frontal view; (6) head, dorsal view.
Figures 7–11. Sarcophaga (Liopygia) argyrostoma (R.-D.), female (photo by P. Ślipiński). (7) Dorsal view; (8) lateral view; (9) head, frontal view; (10) head, right lateral view; (11) head, dorsal view.
Figures 12–15. Sarcophaga (Liopygia) argyrostoma (R.-D.), female (photo by P. Ślipiński). (12) Abdomen, ventral view; (13) terminalia, ventral view; (14) receptacula seminis, A – spermathecal duct; (15) mid femur, o – femoral organ, right lateral view.
Figures 16–22. Sarcophaga (Liopygia) argyrostoma (R.-D.), first instar larva (photo by K. Szpila). (16) Anterior end of body, antero-lateral view; (17) anterior end of body, ventral view; (18) maxillary palpus; (19) ventral organ; (20) antennal complex; (21) functional mouth orifice; (22) Kellins organ on the first thoracic segment. Abbreviations: AN – antennal complex; AS1 – first additional sensillum coeloconicum; AS2 – second additional sensillum; CI – cirri; ES – elongated sensillum; LL – labial lobe; LO - labial organ; MH – mouthhook; MO – functional mouth orifice; MP – maxillary palpus; OR – oral ridges; SB1 – first sensillum basiconicum; SB2 – second sensillum basiconicum; SB3 – third sensillum basiconicum; SC1 – first sensillum coeloconicum; SC2 – second sensillum coeloconicum; SC3 – third sensillum coeloconicum, VO – ventral organ.
Figures 23–28. *Sarcophaga (Liopygia) argyrostoma* (R.-D.), first instar larva (photo by K. Szpila). (23) Fourth abdominal segment, dorsal view; (24) third abdominal segment, lateral view, lateral papilla; (25) third abdominal segment, ventral view; (26) third abdominal segment, spinulation, anterior spinose band, ventral surface; (27) posterior end of body, ventral view; (28) posterior end of body, posterior view. Abbreviations: AO – anal opening; AP – anal papilla; CR – transverse crevice; HLS – hair-like spines; LCV – lateral creeping welt; P1 – dorsal papilla; P2 – subdorsal papilla; P3 – supralateral papilla; P4 – infralateral papilla; P5 – subventral papilla; P6 – ventral papilla; P7 – supraventral papilla; PS – posterior spiracles; SC – spiracular cavity; VCV – ventral creeping welt.
Figures 29–36. *Sarcophaga (Liopygia) argyrostoma* (R.-D.), first instar larva (photo by P. Slipiński). (29) Habitus, lateral view; (30) habitus, dorsal view; (31) posterior end of body; (32) posterior spiracles, with long fore chamber; (33) posterior spiracles; (34) posterior spiracles, enlarged; (35) anterior part of body; (36) anterior part of body, cephaloskeleton, lateral view. Abbreviations: AI–AVII – abdominal segments; AD – anal division; DC – dorsal cornua; IS – intermediate sclerite; MH – mouthhooks; PB – parastomal bar; Pn – pseudocephalon; TI– TIII – thoracic segments; VC – ventral cornua; VP – ventral plate.
Figures 37–46. *Sarcophaga* (*Liopygia*) *argyrostoma* (R.-D.), intermediates between instars I and II (photo by P. Ślipiński). (37) Anterior part of body, cephaloskeleton, lateral view; (38) habitus, lateral view; (39) anterior part of body, cephaloskeleton, lateral view; (40) anterior spiracle; (41) posterior spiracles; (42) habitus, lateral view; (43) anterior part of body, lateral view; (44) habitus, dorsal view; (45) anterior part of body, lateral view; (46) posterior part of body with posterior spiracles. Abbreviations: AS – anterior spiracle; MH1 – mouthhook LI, MH2 – mouthhook LII.
Figures 47–52. Sarcophaga (Liopygia) argyrostoma (R.-D.), second instar larva (photo by P. Ślipiński). (47) Habitus, lateral view; (48) habitus, lateral view, internal organs AS, C, PS visible; (49) anterior part of body, cephaloskeleton, lateral view, with wide collar of spines; (49a) anterior spiracle; enlarged; (50) posterior part of body, lateral view, with posterior spiracle and anal pad; (51) posterior spiracles, with spiracular cavity; (52) posterior spiracles. Abbreviations: AD – anal division; AS – anterior spiracle, C – cephaloskeleton, DB – dorsal bridge; DC – dorsal cornua; DS – dental sclerite; IS – intermediate sclerite; MH – mouthhooks; PB – parastomal bar; PS - posterior spiracles, VC – ventral cornua; VP – ventral plate.
Figures 53–60. *Sarcophaga (Liopygia) argyrostoma* (R.-D.), second instar larva (photos 53, 54 by M. Kowalewska-Groszkowska, photos 55–57 by K. Szpila, the others by M. Roszkowska). (53) Habitus, lateral view; (54) anterior part of body, lateral view; (55) anterior part of body, antero-ventral view; (56) anterior end, functional mouth opening, ventral view; (57) ventral organ, ventral view; (58) anterior spiracles, lateral view; (59) two branches, enlarged; (60) maxillary palpus, anterior view. Abbreviations: MP – maxillary palpus; VO – ventral organ.
Figures 61-67. Sarcophaga (Liopygia) argyrostoma (R.-D.), second instar larva (photo by M. Roszkowska, the others by K. Szpila). (61) Fourth abdominal segment, ventral view; (62) ventral spines on fourth abdominal segment; (63) spines and processes on third thoracic segment; (64) posterior part of body with anal pad, posterior view; (65) posterior part of body with anal pad, postero-lateral view; (66) posterior part of body with anal pad, postero-ventral view; (67) posterior spiracles, posterior view. Abbreviations: Ao – anal opening; S – slit; PSH – posterior spiracular hairs; P1–P6 – papillae; Pa – anal papilla.
Figures 68–74. Sarcophaga (Liopygia) argyrostoma (R.-D.), third instar larva (photos 68, 69, 74 by P. Ślipiński, the others by K. Szpila). (68) Habitus lateral view; (69) cephaloskeleton, lateral view; (70) anterior part of body, lateral view; (71) anterior spiracle; (72) integumental spines; (73) posterior spiracles, normal position; (74) posterior spiracles, separation position.
Figures 75–82. *Sarcophaga (Liopygia) argyrostoma* (R.-D.), third instar larva (photos 75–77 by M. Roszkowska and photos 78–82 by M. Kowalewska-Groszkowska). (75) Anterior part of body, antero-lateral view; (76) anterior part of body, antero-ventral view; (77) maxillary palpus and antennal complex; (78) anterior part of body, frontal view; (79) anterior part of body, dorsal view; (80) anterior part of body, ventral view; (81) habitus, anterior part, ventral view; (82) habitus, posterior part, ventral view. Abbreviations: AN – antennal complex, CI – cirri, LL – labial lobe, MH – mouthhook, MO – functional mouth opening, MP – maxillary palpus.
Figures 83–90. *Sarcophaga (Liopygia) argyrostoma* (R.-D.), third instar larva (photo by K. Szpila). (83) Maxillary palpus, anterior view; (84) anterior spiracle, lateral view; (85) fourth abdominal segment, dorsal view; (86) dorsal spines on fourth abdominal segment; (87) second abdominal segment, ventral view; (88) ventral spines on second abdominal segment; (89) posterior part of body with anal pad, posterior view, P1–P7; (90) posterior part of body with anal pad, postero-lateral view.
Figures 91–98. *Sarcophaga (Liopygia) argyrostoma* (R.-D.), third instar larva (photos 91–93 by K. Szpila, photos 94–96 by M. Kowalewska-Groszkowska, photos 97, 98 by M. Roszkowska). (91) Posterior lower papilla, P7, postero-lateral view; (92) posterior end of body with anal pad, ventral view; (93) posterior spiracles, posterior view; (94) anal division with anal pad, posterior view, P1–P7; (95) left anal papilla enlarged; (96) right anal papilla enlarged; (97) papillae, spines and integument perforation on ventral side of TIII; (98) widenings and lateral processes on abdominal segments I–III.
Figures 99–106. *Sarcophaga (Liopygia) argyrostroma* (R.-D.), puparium (photos 99, 100 by P. Ślipiński, the others by M. Kowalewska-Groszkowska). (99) Dorsal view; (100) lateral view; (101) dorsal view; (102) lateral view; (103) anterior part, anterior view; (104) anterior part, with anterior spiracles 3rd instar, anterior view, enlarged; (105) empty puparium, lateral view; (106) part of abdominal segments VI, VII and anal division LIII, lateral view.
Figures 107–114. *Sarcophaga* (*Liopygia*) *argyrostroma* (R.-D.), puparium (photo by M. Kowalewska-Groszkowska). (107) Posterior end, dorsal view; (108) posterior end, ventral view; (109) posterior end, posterior view; (110) third papilla, lateral view; (111) second papilla, posterior view; (112) arrangement of spines on anterior and posterior margin of segment, processes, papillae and perforations on VI abdominal segment of L III; (113) enlarged sensilla from VI abdominal segment, dorsal view; (114) upper flap of puparium with anterior spiracula of LIII, dorsal view.
Figures 115–120. Sarcophaga (Liopygia) argyrostoma (R.-D.), puparium (photo by M. Kowalewska-Groszkowska). (115) Upper flap of puparium with anterior spiracula of LIII, lateral view; (116) anterior spiraculum of LIII, enlarged, round orifices visible, lateral view; (117) lower flap of puparium, lateral view; (118) lower flap of puparium, ventral view; (119) lower flap of puparium, internal view, attached cephaloskeleton visible; (120) posterior end, with posterior spiracles, posterior view.
Figure 121. Graphical representation of *Sarcophaga (Liopygia) argyrostoma* (R.-D.) DNA barcode.