

Skimming behaviour and spreading potential of *Stenus* species and *Dianous coerulescens* (Coleoptera: Staphylinidae)

Carolin Lang · Karlheinz Seifert · Konrad Dettner

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Abstract Rove beetles of the genus *Stenus* Latreille and the genus *Dianous* Leach possess pygidial glands containing a multifunctional secretion of piperidine and pyridine-derived alkaloids as well as several terpenes. One important character of this secretion is the spreading potential of its different compounds, stenusine, norstenusine, 3-(2-methyl-1-butenyl)-pyridine, cicindeloin, α -pinene, 1,8-cineole and 6-methyl-5-heptene-2-one. The individual secretion composition enables the beetles to skim rapidly and far over the water surface, even when just a small amount of secretion is emitted. Ethological investigations of several *Stenus* species revealed that the skimming ability, skimming velocity and the skimming behaviour differ between the *Stenus* species. These differences can be linked to varied habitat claims and secretion saving mechanisms. By means of tensiometer measurements using the pendant drop method, the spreading pressure of all secretion constituents as well as some naturally identical beetle secretions on the water surface could be established. The compound 3-(2-methyl-1-butenyl)pyridine excelled stenusine believed to date to be mainly responsible for skimming relating to its surface activity. The naturally identical secretions are not subject to synergistic effects of the single compounds concerning the spreading potential. Furthermore,

evolutionary aspects of the Steninae's pygidial gland secretion are discussed.

Keywords *Stenus* · Skimming behaviour · Skimming velocity · Spreading potential · Spreading pressure · Evolution

Introduction

In 1774, Franklin (in Gaines 1966) observed first the phenomenon of spreading as he put a teaspoon of oil on the water surface of a pond. The ability of the oil to spread on the water surface forming a thin layer he called "spreading". With help of the applied amount of oil and the oil-covered area of the pond, he estimated that the layer must be monomolecular.

Nowadays, spreading is defined generally as the expansion of a fluid or solid phase on a fluid surface establishing a monolayer (Dörfler 1994). However, the phenomenon of spreading can not only be found in physical chemistry science of surfaces but also in the great outdoors. Small staphylinid beetles of the genera *Stenus* and *Dianous* use spreading as an effective and extraordinary way of locomotion on water surfaces.

Rove beetles of the genera *Stenus* Latreille and *Dianous* Leach are small, slender and black insects that predominantly inhabit wet biotopes, e.g. marshes, banks of pools and rivers (Horion 1963; Dettner 1987). The genus *Stenus* is one of the species-rich genera within the animal kingdom. Till this day, 2,377 species and eight fossil species are known worldwide (Puthz 2008).

The direct neighbourhood of open waters in the habitat of most *Stenus* and *Dianous* species harbours danger for the beetles. While hunting for springtails, it is possible that the hydrophobic beetles accidentally fall into water. The unusual

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C. Lang (✉) · K. Dettner
Department of Animal Ecology II, University of Bayreuth,
95440 Bayreuth, Germany
e-mail: carolin.lang@uni-bayreuth.de

K. Seifert
Department of Organic Chemistry, University of Bayreuth,
95440 Bayreuth, Germany

behaviour of spreading has evolved in these insects as a mechanism to save themselves from drowning and to escape predators like water striders (Linsenmair 1963; Schildknecht et al. 1975). For the first time, Piffard (1901) discovered that some species of *Stenus* beetles are able to move over the water surface in a rapid and extraordinary manner. At this, the beetle glides on the water with high velocity without using its legs. A few years later, Billard and Bruyant (1905) observed this locomotion in *Stenus tarsalis* Ljungh and *Stenus cicindeloides* Schaller. They report an emission of chemicals that interact with the water surface like a surfactant and propel the beetle fast forward comparable to a “soap boat” (Fig. 1). This locomotion is driven by the marangoni propulsion (Scriven and Sternling 1960). Marangoni flows are those forced by surface tension gradients. Surfactants like soap and the emitted substances of the beetles are molecules that find it energetically favourable to reside at the free surface and act to decrease the local surface tension (Bush and Hu 2006).

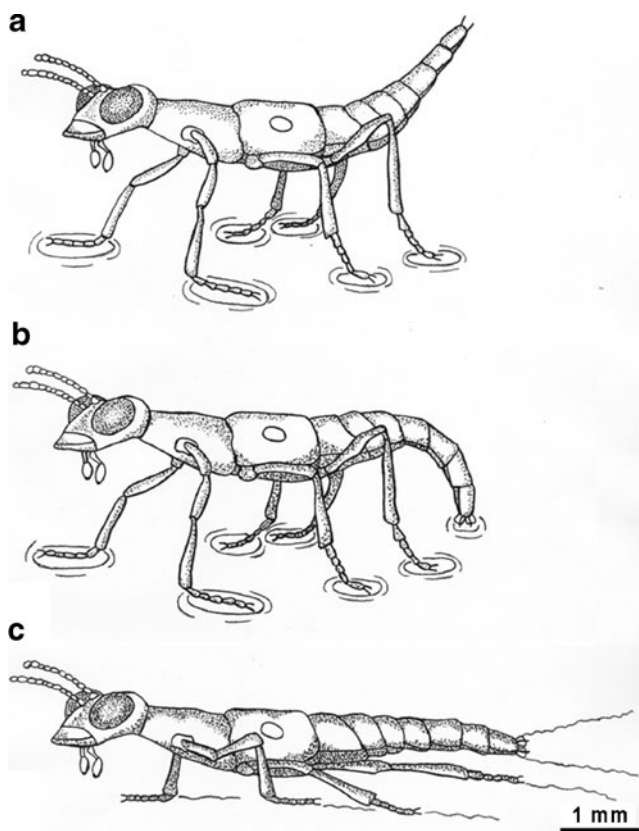


Fig. 1 Skimming procedure of *Stenus comma*. **a** Fallen on the water surface, the beetle is carried by the surface tension due to the hydrophobicity of its tarsi. **b** The beetle bends its abdomen and emits a small amount of its gland secretion on the water surface. **c** Lying flat on the water surface due to reduction of surface tension, the beetle is propelled fast forward by the spreading agents in its secretion. Middle and hind legs are held close to the body. The skimming direction can be changed by bending the abdomen

Jenkins (1960) investigated the spreading behaviour of *Stenus* and *Dianous* beetles. The spreading action of these two genera he named “skimming”. Besides, he observed the negative phototactic navigation of *Stenus* and *Dianous* on the water surface towards the dark bank of a pool. The beetles waste no time to achieve the save waterside. Schildknecht et al. (1975) found that a secretion of the pygidial glands in the anal region is responsible for the skimming action (Fig. 2). The secretion is emitted by the everted glands as soon as the beetle contacts the water surface and spreads on the water surface immediately.

The gland compounds form a monomolecular film whose front pushes the beetle forward (Dettner 1991). By this kind of locomotion, e.g. *Stenus comma* can achieve a velocity of 0.75 ms^{-1} , and if the secretion is continuous, a distance up to 15 m can be covered (Linsenmair and Jander 1963). Apart from *Stenus* and *Dianous* beetles, the extraordinary movement is just shown by the water cricket *Velia caprai* Tamarinini, which uses its rostrum and as well spreading active saliva for skimming (Linsenmair and Jander 1963). No other animal is known to have this unique kind of locomotion.

The driving force for skimming is the spreading potential of the gland compounds. Thereby, it is essential that the gland compounds are barely soluble in water; otherwise, it is not possible to form a monolayer. Additionally, the spreading agent has to exhibit a lower surface tension than the layer-carrying substance which is water in case of *Stenus* and *Dianous* (Adamson and Gast 1997).

Schildknecht (1976) discovered that the *Stenus* secretion consists of several different compounds. Stenusine (1) is

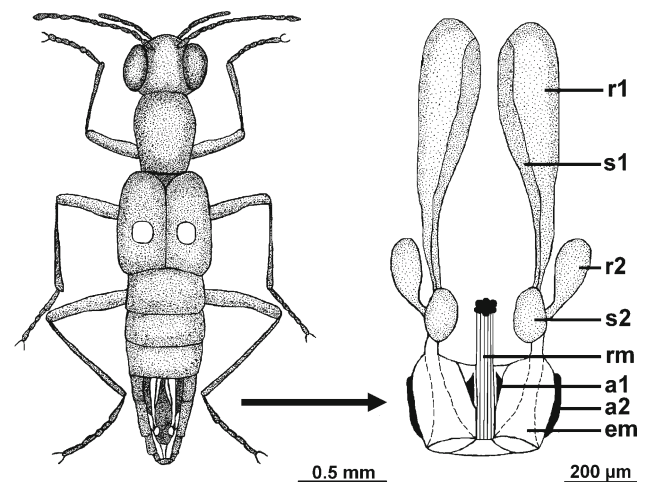


Fig. 2 Pygidial gland system of *Stenus comma*. *Left side* the pair of pygidial glands is arranged in the abdominal tip. *Right side* schematic diagram of the paired pygidial glands in detail. *r1* big reservoir, *s1* secretory cells associated to *r1*, *r2* small reservoir, *s2* secretory cells associated to *s2*, *rm* retractor muscle of eversible membrane parts, *a1* and *a2* apodemes on which retractor muscles insert, *em* eversible membrane parts forming basal pouches; from Szujewski 1961, Whitman et al. 1990 and Schierling unpublished, modified

mainly responsible for skimming because it is featured by the highest spreading pressure and therefore lowers the surface tension of water the most compared with 1,8-cineole (6) and 6-methyl-5-heptene-2-one (7) (Schildknecht et al. 1976). Besides, 1 forms the main component of the secretion of most *Stenus* species. In the course of time, many more gland compounds were identified characterizing the gland content (Fig. 3). The alkaloids 1 (Schildknecht et al. 1975) and norstenusine (2) (Kohler 1979), the pyridine compound 3-(2-methyl-1-butenyl)pyridine (3) (Lusebrink et al. 2009) and the epoxy piperidine cicindeloin (4) (Müller et al. 2012) are stored in the big reservoirs, whereas the terpenoids α -pinene (5) (Neumann 1993), 6 and 7 (Schildknecht 1970) are provided in the small reservoirs (Fig. 2).

The spreading pressures of the compounds can be measured experimentally for instance at an interfacial tensiometer (Schildknecht et al. 1976). After the measurement of the surface tension σ of the compounds against air and the interfacial tension γ against water, the spreading pressure P can be calculated according to the following equation defined by Wolf (1957)

$$P = \sigma_{\text{water}} - (\sigma_{\text{substance}} + \gamma_{\text{substance/water}}) \quad (1)$$

Spreading action can only be observed if the difference of σ_{water} and $\sigma_{\text{substance}} + \gamma_{\text{substance/water}}$ is positive, that is, that work is obtained (Wolf 1957). Otherwise, the applied liquid remains as a lens on the water surface.

Although almost all Steninae and representatives of *Dianous* contain the spreading-active alkaloid 1 in their pygidial glands, not every species exhibits skimming behaviour (Jenkins 1960; Linsenmair 1963; Dettner 1991). It is assumed that the ability to skim can be linked to the different kinds of habitats that are colonised by *Stenus* (Jenkins 1960). In this study, we investigate several *Stenus* species with reference to their ability to skim, the skimming behaviour and the skimming velocity. In addition, we present a modified method of measurement of the spreading potential of the secretion compounds. As quite recently all up to now

known secretion compounds (1–7) are synthetically available, it is first possible to measure the spreading potentials of all known compounds. Over and above, we mixed artificially natural identical secretions of some *Stenus* species to prove their surface activity.

Materials and methods

Collection, determination and keeping of the beetles

The *Stenus* beetles were collected in summer 2011 at a mountain stream in the Black Forest, Germany, near Baiersbronn (48°51'00"N; 8°31'41"E; collected species: *D. coerulescens*, *Stenus stigmula*) and at a lakeside near Creußen, Germany (49°52'17"N; 11°36'40"E; collected species: see all species except of *D. coerulescens* and *S. stigmula* listed in Table 1). The small lake features a large marshy accretion zone with plant debris. After collection, the beetles were identified by using the key of Lohse (1964), the additions of Lohse (1989) and additionally the new identification key of Puthz (2001, 2008). After determination, the beetles were kept in Petri dishes on moist gypsum mixed with activated charcoal to prevent mould formation and to provide enough moisture. The gypsum was sprayed with water once a week. The beetles were fed twice a week ad libitum with wing-deformed mutants of *Drosophila melanogaster* flies and kept under standardized conditions in a climate chamber (20 °C; 16:8-h light/dark photoperiod).

Determination of skimming velocities and behavioural investigations

The skimming velocity of nine *Stenus* species and *Dianous coerulescens* was established by a video camera (JVC Professional CCD), which was connected to a TV and an automatic code timer (RTV-920 HiFi). Three hours before the beetles were filmed, they were put carefully in small glass jars in order to calm the beetles before filming, preventing disturbance and unnecessary emission of their pygidial secretion. The skimming

Fig. 3 The pygidial gland secretion compounds of *Stenus* selected for this study (Schildknecht 1970; Schildknecht et al. 1975; Kohler 1979; Neumann 1993; Lusebrink et al. 2009; Müller et al. 2012): stenusine (1), norstenusine (2), 3-(2-methyl-1-butenyl)pyridine (3), cicindeloin (4), α -pinene (5), 1,8-cineole (6) and 6-methyl-5-heptene-2-one (7)

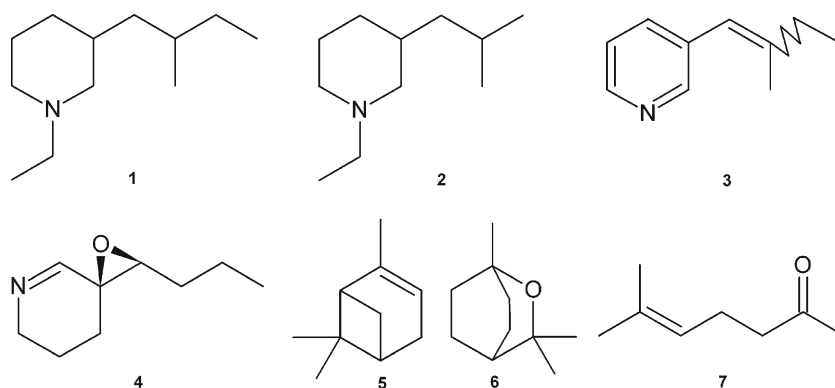


Table 1 Skimming ability of different *Stenus* species and *D. coeruleus*

Species	Subgenus	1	2	3	4	5	6	7	Skimming
<i>S. bifoveolatus</i> Gyllenhal 1827 ^a	<i>Metatesnus</i>	++	++	–	–	–	+	–	Yes
<i>S. biguttatus</i> Linnaeus 1758 ^a	<i>Stenus</i>	++	++	–	–	+	+	+	Yes
<i>S. bimaculatus</i> Gyllenhal 1810 ^a	<i>Stenus</i>	++	+	–	–	+	+	–	Yes ^c
<i>S. binotatus</i> Ljungh 1804 ^a	<i>Metatesnus</i>	+	+	++	+	–	–	–	Yes
<i>S. boops</i> Ljungh 1804 ^b	<i>Stenus</i>	++	+	–	–	+	+	–	No
<i>S. cicindeloides</i> Schaller 1783 ^b	<i>Hypostenus</i>	+	+	+	+	–	–	–	Yes
<i>S. circularis</i> Gravenhorst 1806 ^b	<i>Stenus</i>	++	++	–	–	+	+	–	No
<i>S. clavicornis</i> Scopoli 1763	<i>Stenus</i>	++	+	–	–	+	+	–	No
<i>S. comma</i> LeConte 1863 ^a	<i>Stenus</i>	++	+	–	–	+	++	+	Yes
<i>S. flavipalpis</i> Thomas 1860 ^a	<i>Hemistenus</i>	++	+	–	–	+	–	–	No
<i>S. flavipes</i> Stephens 1833 ^a	<i>Metatesnus</i>	++	–	–	–	–	–	–	Yes
<i>S. fossulatus</i> Erichson 1840 ^b	<i>Stenus</i>	++	+	–	–	+	+	–	Yes ^c
<i>S. fulvicornis</i> Stephens 1833 ^a	<i>Hypostenus</i>	++	+	–	–	–	+	–	Yes
<i>S. humilis</i> Erichson 1839 ^a	<i>Stenus</i>	++	+	–	–	+	–	–	No
<i>S. impressus</i> Germar 1824 ^b	<i>Hemistenus</i>	++	+	–	–	+	+	–	No
<i>S. juno</i> Paykull 1800 ^a	<i>Stenus</i>	++	+	–	–	–	+	–	No
<i>S. latifrons</i> Erichson 1839 ^a	<i>Hypostenus</i>	++	+	–	–	–	+	–	Yes
<i>S. melanarius</i> Stephens 1833 ^b	<i>Stenus</i>	++	+	–	–	–	–	–	No
<i>S. nitidiusculus</i> Stephens 1833 ^a	<i>Metatesnus</i>	++	+	–	–	–	+	–	Yes ^c
<i>S. picipes</i> Stephens 1833 ^b	<i>Metatesnus</i>	++	+	–	–	+	+	–	Yes
<i>S. providus</i> Erichson 1839 ^b	<i>Stenus</i>	++	+	–	–	+	+	–	No
<i>S. pubescens</i> Stephens 1833 ^a	<i>Metatesnus</i>	++	+	+	–	–	–	–	Yes ^c
<i>S. similis</i> Herbst 1784 ^a	<i>Hypostenus</i>	++	+	++	–	+	+	–	Yes
<i>S. solutus</i> Erichson 1840 ^a	<i>Hypostenus</i>	+	+	++	+	+	+	–	Yes
<i>S. stigmula</i> Erichson 1840	<i>Stenus</i>	++	+	–	–	–	–	–	Yes
<i>S. tarsalis</i> Ljungh 1804 ^a	<i>Hypostenus</i>	++	+	++	–	–	–	–	Yes
<i>D. coeruleus</i> Gyllenhal 1810 ^a	Genus <i>Dianous</i>	++	+	–	–	+	+	–	Yes

++ large quantity of substance, + substance present, – no substance present, 1 stenusine, 2 norstenusine, 3 3-(2-methyl-1-butenyl)pyridine, 4 cicindelone, 5 α -pinene, 6 1,8-cineole, 7 6-methyl-5-heptene-2-one

^aInvestigated by Schierling et al. (submitted for publication)

^bInvestigated by Lusebrink (2007)

^cSkimming ability observed by Betz (1999)

observations were conducted in a round trough (diameter 24.1 cm) filled with tap water. In the middle of the trough, a small floater made of cork was affixed. This floater could be sunk manually. The beetles were shaken out of the glass jars via a Teflon[®]-coated cone on the floater without touching or squeezing the beetles because of possible loss of secretion. Only when the beetles did not skim voluntarily, the floater was sunk to force the beetles to skim. After every test, the water in the trough was changed to remove the remaining secretion on the water surface that could influence the following test. The films were analysed in 200th seconds steps, and the skimming distance was measured. Only absolute skimming distances without leg action were noted. The velocity was calculated using the following equation:

$$v = \frac{d \cdot \left(\frac{D_{\text{real}}}{D_{\text{screen}}} \right)}{(t_{\text{start}} - t_{\text{end}})} \quad (2)$$

v velocity (in centimetres per second)

d distance (in centimetres)

D_{real} diameter of the trough (in centimetres)

D_{screen} diameter of the trough on the TV screen (in centimetres)

t_{start} beginning of the skimming action (in seconds)

t_{end} stopping of the skimming action (in seconds)

Statistical comparisons of the skimming velocities of the investigated species were conducted using the non-parametric Kruskal–Wallis H test and the corresponding post hoc procedure in terms of the Tukey–Kramer test (Siegel and Castellan 1988) implemented in Statistica 8 (StatSoft Inc. 1984–2004, Tulsa, OK, USA).

Quantification of the natural secretion of selected species

Some of the collected beetles (40; e.g. ten beetles per the four following species) were used for quantitative analysis of the single compounds proportionate in their natural secretion by using gas chromatography–mass spectrometry (GC–MS). Representatives of *Stenus clavicornis* Scopoli, *S. comma* LeConte, *Stenus similis* Herbst and *S. solutus*

Erichson were killed by freezing to -20° . Then the glands were dissected (10 gland systems per every species and vial) and transferred for extraction into conical glass vials containing 30 μ l ethylacetate (Suprasolv, Merck, Germany). The gland specimens were then homogenized by sonication for 10 min. The quantitative analyses were performed on a Finnigan MAT GCQ ion trap GC–MS coupling instrument using a BPX-5 column (SGE, 25 m \times 0.22 mm i.d.). The temperature program was from 50 $^{\circ}$ C (hold 2 min) at 10 $^{\circ}$ C/min to 280 $^{\circ}$ C, and the carrier gas was helium (1 ml/min). To every extract, a quantification standard was added, and then 0.5 μ l of the prepared extract injected into the GC–MS. For quantification, the obtained TIC peaks were electronically integrated and compared to prior developed calibration files by the software delivered with the GC–MS (GCQ Data Processing 2.31, Finnigan Corp.). As isopiperitenol and 3-(1-isobutenyl)pyridine could not be detected or just as a minor compound while quantification, they were disregarded concerning this study. Furthermore, the acquisition of these compounds held difficulties.

Measurement of spreading pressures at the tensiometer

Measurements of the spreading pressures were performed on a drop volume tensiometer (TVT 1 Lauda; Dr. R. Wobser GmbH + CoKG) gratefully provided by Prof. H. Hoffmann, Bayreuther Zentrum für Kolloide und Grenzflächen, University of Bayreuth, Germany. In this study, we used the pendant drop method to measure the surface tension of an oversaturated aqueous solution of every secretion compound (1–7). Before the measurements, the adequate concentration of the oversaturated solution of **1** was empirically determined by serial dilution. At the adequate concentration, the tensiometer delivered correct mean values of the surface tension. All other specimens were measured at the same concentration to achieve comparable values. It was important to measure the surface tension of water before every new measurement of a specimen. Authentic compounds (1–4) were not commercially available, so were synthesized by the workgroup of Prof. K. Seifert, University of Bayreuth, Germany. The other compounds (5–7) were purchased from Sigma-Aldrich, St. Louis, MO, USA. The natural identical secretions were mixed equivalently to the single compound solutions regarding the quantification results. To calculate the spreading pressure of the measured specimens, a modified version of Eq. (1) was used. As we measured the surface tension of oversaturated solutions, the interfacial tension γ can be omitted. The spreading pressure P is due to the difference of the surface tension of clear water and the surface tension of the oversaturated solution:

$$P = \sigma_{\text{water}} - \sigma_{\text{oversaturatedSolution}} \geq 0 \quad (3)$$

We proved this assumption by a threefold test measurement with octanole, which also exhibits surface activities.

At first, P was measured and calculated using Eq. (1), and then we determined P with Eq. (3). The results varied scarcely. This method harboured the advantage that a very small quantity on milligram scale of the authentic material was required.

Results

Observed skimming ability of *Stenus* species and *D. coerulea*

Stenus species belonging to different subgenera according to Puthz (2001, 2008) and *D. coerulea* were investigated with reference to their skimming ability. In Table 1, the results and the corresponding gland compounds are summarized.

Skimming behaviour and skimming velocity

Ten skimming species listed in Table 1 were used for statistical analysis and closer examination of behaviour while skimming. It became evident that the skimming behaviour is species-specific and can be characterized by variable features. While some species show a voluntary straightforward locomotion to achieve the side of the trough in which the skimming action was performed, other species skim stepwise probably due to interrupted secretion release. A few species had to be forced to skim in order to obtain usable values. In the following, the different skimming behaviour of nine *Stenus* species and *D. coerulea* investigated is summarized (Table 2). Not only has the skimming behaviour significantly differed between the species but also the skimming velocities determined (Fig. 4). Generally, species exhibiting continuous secretion emission and voluntary skimming (e.g. genus *D. coerulea*, *S. comma*, *Stenus biguttatus* and *S. stigmula*) reveal a higher skimming velocity than species with fitful and involuntary skimming (e.g. *Stenus flavipes*, *Stenus fulvicornis* and *S. tarsalis*; Fig. 4).

Furthermore, the skimming velocities determined were statistically investigated as well. Between the ten species, highly significant differences can be demonstrated (Kruskal–Wallis H test; H (degrees of freedom=9, $n=233$)=144.67, $p<0.001$). Also the post hoc procedures (Tukey–Kramer test) often revealed highly significant differences between many species (indicated by lower case in Fig. 4).

Spreading pressures of the secretion compounds

Beside ethological investigations of skimming *Stenus* species, also physicochemical properties regarding the spreading potential of the secretion compounds were addressed in this study. To determine the spreading

Table 2 Different skimming behaviour of *D. coerulescens* and nine *Stenus* species

Species	Skimming behaviour
<i>D. coerulescens</i>	Always voluntary skimming; straightforward skimming action (“linear skimming”) with highest velocities; continuous secretion release
<i>S. biguttatus</i>	Always voluntary skimming; straightforward skimming action with high velocities; continuous secretion release
<i>S. comma</i>	
<i>S. stigmula</i>	
<i>S. flavipes</i>	Not always voluntary skimming and not always straightforward; discontinuous secretion release and therefore fitful skimming action; while skimming the beetles often turn around their own axis very fast (“circular skimming”)
<i>S. latifrons</i>	Often voluntary and mostly straightforward skimming; continuous skimming but the velocities vary strongly between the tested individuals
<i>S. fulvicornis</i>	Skims not always voluntarily and not always straightforward; discontinuous secretion release and therefore fitful skimming action
<i>S. tarsalis</i>	Skims always involuntarily and straightforward but comparatively slowly; often a lateral drift often can be observed; discontinuous secretion release
<i>S. similis</i>	
<i>S. solutus</i>	If rarely skimming action can be observed, the beetles skim always involuntarily and very slowly; the beetles rather prefer to swim with leg action to the side of the trough

pressures of every single secretion compound (1–7), an aqueous oversaturated solution was assembled for measurement at the drop volume tensiometer. Differences of spreading pressures between the substances could be revealed (Table 3). Most substances showed high surface activity. The monoterpene compound α -pinene (5) is characterized by the highest spreading pressure followed by 3-(2-methyl-1-butenyl)pyridine (3), norstenusine (2), stenusine (1) and 6-methyl-5-hepten-2-one (7). Cicindeloine (4) and the monoterpene 1,8-cineole (6) are least surface active.

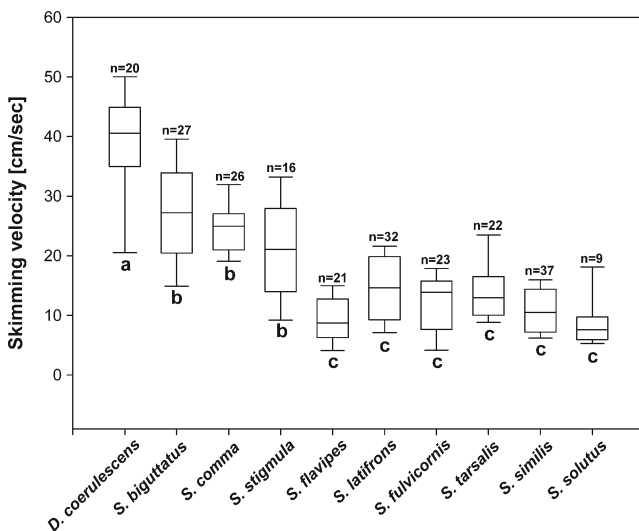


Fig. 4 Skimming velocities of nine *Stenus* species and *D. coerulescens*. The boxplots are shown with median and standard deviation bars. *D. coerulescens* reveals the highest velocity, followed by *S. biguttatus*, *S. comma* and *S. stigmula*. The remaining species cannot be distinguished statistically significantly. Lowercase letters a, b and c indicate significant differences depending on *p* values; for exact statistical parameters (all *p* values), refer to Online Resource Table S1

For investigation of naturally identical secretion compositions at the tensiometer, four beetle species were chosen based on their gland content. The species typify representatives of different *Stenus* groups: *S. clavicornis*, a species that exhibits no skimming behaviour, is characterized by a stenusine-based secretion composition (piperidine group; Schierling et al., submitted for publication). In addition, a small amount of 2 and 6 can be found in the glands of *S. clavicornis*. The monoterpenes 5 and 6 are present as unquantifiable trace compounds. The second species chosen, *S. comma*, also belonging to the piperidine group, skims voluntarily and with high velocities. *S. comma* also possesses a 1-based secretion, but the amount of 2 and 6

Table 3 Spreading pressures (\pm SEM) of all tested secretion compounds 1–7 measured at the drop volume tensiometer (mean values of threefold measurements)

Substance	Spreading pressure (30 °C) (mN m^{-1})	Spreading pressure (19 °C) ^b (dyn cm^{-1})
1	15.89	36.3
2	17.90	–
3	22.22	–
4	4.27 ^a	–
5	24.17	–
6	9.88	29.2
7	15.63	32.4

Unit dynes per centimetre equates to millinewtons per metre

1 stenusine, 2 norstenusine, 3 3-(2-methyl-1-butenyl)pyridine, 4 cicindeloine, 5 α -pinene, 6 1,8-cineole, 7 6-methyl-5-hepten-2-one, – not measured by Schildknecht et al. (1975)

^a Regard with reservation due to the extreme low solubility of 4 in water and the resinous consistency

^b Measured by Schildknecht et al. (1975)

in the secretion is numerically higher than in the glands content of *S. clavicornis*. The substances **5** and **7** are again unquantifiable trace compounds. *S. similis* represents a moderate skimming species. The gland content is based on **1**, also **2** and **6** can be found as minor compounds. After **1**, the pyridine-derived compound **3** captures the second largest amount of the *S. similis* secretion (pyridine group; Schierling et al., submitted for publication). The fourth chosen *Stenus* species, *Stenus solutus*, shows just rare skimming action. *S. solutus* possesses **1** just as a minor compound that could not be quantified. The secretion is mainly composed of **3** and **4** (epoxypiperidine group; Schierling et al., submitted for publication).

Based on the results summarized in Table 4, naturally identical beetle secretions were mixed, and the spreading pressures were determined at the tensiometer (Table 5). The measurements revealed no differences of the spreading pressure values of the synthetic secretions of *S. clavicornis*, *S. comma* and *S. similis*. Only the secretion composition of *S. solutus* exhibits the least surface activity and therefore the least spreading pressure.

Discussion

Habitat claims influence skimming behaviour

In this study, we investigated the skimming ability and the skimming behaviour of several *Stenus* species under laboratory conditions. Not all Steninae exhibit the unique skimming behaviour to move rapidly and in an extraordinary manner over the water surface (Table 1) as already reported by Jenkins (1960), Linsenmair (1963) and Dettner (1991). In addition, skimming ability is restricted to some subgenera in *Stenus*. While several representatives of the genus *Dianous*, the subgenus *Stenus s. str.* like *S. comma* (Jenkins 1960; Linsenmair and Jander 1963) and representatives of the subgenus *Hypostenus* such as *S. cicindeloides* and *S. tarsalis* (Billard and

Table 4 Quantitative composition (%±SEM) of pygidial gland secretions of four chosen *Stenus* species determined with the help of a calibration curve and following peak area integration at the GC–MS system

Species	1	2	3	4	5	6	7
<i>S. clavicornis</i>	95.9	3.6	–	–	<i>n</i>	0.5	–
<i>S. comma</i> ^a	82.3	12.2	–	–	<i>n</i>	5.5	<i>n</i>
<i>S. similis</i> ^a	73.8	0.8	23.6	–	<i>n</i>	1.8	<i>n</i>
<i>S. solutus</i> ^a	<i>n</i>	–	24.5	75.5	–	–	–

For chemical structures, see Fig. 3

1 stenusine, **2** norstenusine, **3** 3-(2-methyl-1-butenyl)pyridine, **4** cicindeloine, **5** α -pinene, **6** 1,8-cineole, **7** 6-methyl-5-heptene-2-one

n amount not quantifiable, – substance not present in glands

^a Quantified by Schierling et al. (submitted for publication)

Table 5 Spreading pressures (± SEM) of formulations mixed equivalently to natural secretion compositions of four chosen *Stenus* species determined at the drop volume tensiometer (mean values of threefold measurements)

Species	Spreading pressure (30 °C) (mNm ⁻¹)
<i>S. clavicornis</i>	20.67
<i>S. comma</i>	19.71
<i>S. similis</i>	20.97
<i>S. solutus</i>	11.19 ^a

^a Regard with reservation due to the extreme low solubility of main constituent **4** in water and the resinous consistency

Bruyant 1905) show skimming behaviour, representatives of the outdated subgenus *Nestus* (now implemented in the subgenus *Stenus* according to Puthz 2008) and members of the subgenus *Hemistenus* never do (Linsenmair 1963). This could also be confirmed in this study with the *Stenus* (*Nestus*) species *Stenus boops*, *Stenus circularis*, *Stenus humilis* and *Stenus melanarius* (Table 1). Also members of the subgenus *Metatesnus* are able to skim, which was shown with the species *Stenus bifoveolatus*, *Stenus binotatus*, *S. flavipes*, *Stenus nitidiusculus*, *Stenus picipes* and *Stenus pubescens*, respectively (Table 1; Betz 1999).

Conspicuously, all Steninae possess spreading active substances in their pygidial glands, but not every *Stenus* species shows skimming behaviour. Members of the outdated subgenus *Nestus* and subgenus *Hemistenus* possess surface active gland substances like **1**, **2**, **5** and **6** (Table 1). Therefore, these beetles should be able to show this extraordinary locomotion due to their secretion composition. It is evident that the presence of skimming behaviour in a *Stenus* species is independent from the beetle's pygidial gland secretion composition. As Steninae inhabit interstices (Horion 1963) as well as banks of open waters (Dettner 1987), the disposition of the beetles to display skimming behaviour could be linked to the different habitat claims of the species.

D. coerulea characterized by the significant highest skimming velocity in this study (Fig. 4) inhabits the immediate neighbourhood of waterfalls, weirs and fast flowing mountain streams with decline (Horion 1963). Therefore, it is essential for the beetles' survival to be able to skim rapidly in the fast moving waters. Consequently, these beetles exhibit distinct skimming behaviour and velocity (Table 2; Fig. 4).

The other *Stenus* species, which display always skimming disposition and high skimming velocities, *S. comma*, *S. biguttatus* and *S. stigma*, can be found in habitats close to banks of stagnant open waters (Horion 1963). These biotopes also require voluntary and persistent skimming action.

Several other *Stenus* species investigated are characterized by a proportionally slow skimming velocity and a less disposition to skim like *S. flavipes* (*Metatesnus*) and representatives of subgenus *Hypostenus* such as *Stenus latifrons*,

S. fulvicornis, *S. tarsalis*, *S. similis* and *S. solutus*. These species can be found predominantly in swampy biotopes and wet places within woods, meadows and marshes. If these beetles live in wet places near open waters, they stay in rotten leaves and other detritus material or in places abundantly covered with vegetation (Horion 1963). Therefore, the beetles rarely accidentally fall in open water, and the occasion to skim is uncommon. This might be a reason for the beetles may have not developed a distinctive skimming behaviour like the investigated species *D. coerulea*, *S. comma* and *S. biguttatus*. The situation is similar to tested *Stenus* species in this study like, e.g. *S. clavicornis* (*Stenus*) and *Stenus* (*Nestus*) species *S. boops*, *S. circularis*, *S. humilis* and *S. melanarius* that never show skimming behaviour. These species especially inhabit mosses of marshes, rotten leaves and detritus (Horion 1963), and therefore, skimming behaviour has not been selected in these types of habitats.

Secretion saving mechanisms as a reason for varied skimming behaviour

As another factor for no distinctive skimming behaviour, some kind of saving mechanism can be seen. Generally, the pygidial glands of the Steninae are originally evolved as defensive glands (Dettner 1991). Almost all staphylinid beetles possess defensive glands (Dettner 1991; Thayer 2005). Due to the fact that staphylinids and also representatives of the genus *Stenus* are characterized by reduced elytra and therefore designed with a freely movable and unprotected abdomen, defensive glands become essential against predator attacks (Dettner 1993). On the one hand, the mobile abdomen grants colonisation of interstices in soil, litter, bark of fungi to staphylinid beetles (Dettner 1993). On the other hand, these predominantly moist habitats increase the danger of infestation by microorganisms. In order to avoid this infestation, *Stenus* displays secretion grooming (Kovač and Maschwitz 1990; Betz 1999) in which the beetle spreads its antimicrobial secretion of the defensive pygidial glands over the entire body surface. It is possible that *Stenus* species living in interstices in plant debris or detritus also use their pygidial gland secretion for secretion grooming than use it for skimming action.

Besides, another secretion saving mechanism other than grooming in varied habitat claims is thinkable. In 1960, Jenkins reported in the context of his ethological studies on *Dianous* and *Stenus* species a negative phototactic skimming behaviour to a dark bank of a pool. Fallen on the water surface, the beetles at once orient into the right direction and waste no time to achieve the safe bank while skimming. These results are supported by the observations of Linsenmair (1963). As *Stenus* beetles are equipped with extraordinary large eyes in relation to their body size and compared with

related staphylinid beetles, it is evident that the visual sense is essential for their orientation. Furthermore, it is imaginable that the beetles not only orient to the right direction but also estimate the distance to the bank. The beetles could be able to balance the distance to the safe bank to the amount of secretion in their glands. Only in exceptional circumstances, the beetles “decide” to use their precious secretion, e.g. complete physical exhaustion or repeated attacks by predators (Linsenmair 1963). These circumstances may explain why skimming velocities of 40–75 cm s^{-1} and a covered distance of up to 15 m (Linsenmair and Jander 1963 for *S. comma*) were not achieved by the beetles in this study. The highest velocity shown by *S. comma* has been 39.98 cm s^{-1} (Fig. 4). This finding is confirmed by the maximal velocity reported by Neumann (1993). The experimental setup used in this study and similar by Neumann (1993) did not technically allow a skimming distance more than 24.1 cm, which could not be sufficient for maximum skimming velocity observed by Linsenmair and Jander (1963). The beetles could also rapidly achieve the safe margin of the trough without using the full capacity of their glands. In this context, total pygidial gland reservoir volume and its size also play an important role by variations of skimming action.

Skimming behaviour is based on physiological properties

The skimming behaviour and the course of motion observed in this study are in accordance with the findings of Betz (1999). The swimming action shown by *S. solutus* also follows the course of motion described by Betz (1999). Linsenmair (1963) described for the representatives of subgenus *Hypostenus* *S. cicindeloides* and for *S. tarsalis* a mixture of skimming and swimming behaviour. Also *S. solutus* similar to *S. cicindeloides* concerning the secretion composition rather displays swimming than skimming. Besides, *S. tarsalis* is characterized by a discontinuous secretion release and thus never achieves the skimming velocities like other *Stenus* species (Linsenmair 1963).

Furthermore, body size and body weight also may influence skimming behaviour. Only *S. flavipes* the smallest beetle investigated features “circular” skimming behaviour (Table 2) before it linearly keeps on skimming in the favoured direction. Due to its less body weight and body size, it is possible that the beetle first must physically stabilize before subsequent linear skimming is performed.

Beyond eye morphology, number of ommatidia and the resultant visual faculty may impact skimming behaviour. As mentioned before *Stenus* species tested in this study inhabit varied biotopes. Different habitat demands require different eye adaptations of beetles concerning hunting behaviour for example (Bauer 1985; Bauer et al. 1998). *Stenus* species living in vegetation possess detritus feature smaller eyes and

a less density of ommatidia, whereas *Stenus* species inhabiting open biotopes with poor vegetation possess larger compound eyes and a high density of ommatidia (Betz 2000). In summary, *Stenus* species tested of vegetation-rich habitats show less precise skimming behaviour than Steninae of open habitats possibly due to less optical capacity.

Physicochemical spreading potential of the secretion and evolutionary aspects

Apart from ethological observations (Table 2), also the spreading potential of the secretion compounds is addressed in this study by measuring the spreading pressure. Schildknecht et al. (1975) already measured the spreading pressure of the substances **1**, **6** and **7**. These values for spreading pressures deviate from the values measured in this study (Table 3). However, the measurement method and the measurement temperature used in this study were altogether different than the method used by Schildknecht et al. (1975). At first, other than in this study, an interfacial tensiometer was used and the spreading pressure was calculated with help of Eq. (1) developed by Wolf (1957). In addition, the spreading pressure was estimated with a film balance developed by Hans Kuhn (Schildknecht 1977). It is apparent that the former measurements lead to aberrations in the results. Anyway, tendencies between the values measured by Schildknecht et al. (1975) and the values measured in this study are perceptible. It is noticeable that the spreading pressures of **3** and **5** are much higher than the spreading pressure of **1** which was postulated as “spreading alkaloid” by Schildknecht et al. in 1975.

Depending on the chemical gland content of different *Stenus* species and their relationship, it is possible to develop a chemosystematic arrangement (Francke and Dettner 2005). In the case of *Stenus*, species whose gland secretion consists predominantly of **1** and **2** and minor components **5**, **6** and **7** (Tables 1 and 4) can be regarded as phylogenetically basal species (“piperidine group” according to Schierling et al., submitted for publication). These species represented as *S. clavicornis* and *S. comma* in this study use **1** and **2** mainly for optionally shown skimming behaviour and as predator avoidance (Connert 1974; Dettner et al. 1996; Hesse 2000). Furthermore, the monoterpenoids **5**, **6** and bisnormonoterpene **7** as pharmacologically active substances are used for antimicrobial protection against bacteria and fungi (Schildknecht et al. 1976) and as an insect repellent (Honda 1983; Blum 1981). Phylogenetically more derived *Stenus* species in this study represented by *S. similis* (“pyridine group” according to Schierling et al., submitted for publication) still possess **1** as main component in its glands, but the “new” compound **3** captures also a large part of the secretion. Not only is the spreading pressure of **3** higher than the pressure of **1** but also the antimicrobial effect against *Bacillus sphaericus*,

Escherichia coli and *Serratia entomophila* (Lusebrink et al. 2009).

The next phylogenetically level in this study is represented by *S. solutus* (“epoxypiperidine group” according to Schierling et al., submitted for publication). This beetle species features neither a remarkable amount of **1** nor **2**, **5**, **6** and **7**, but only **3** and **4**. The substance **3** is used to gain at least minor spreading potential. Besides, **3** also acts as an ant deterrent, as a bactericide and as a fungicide against *Verticillium lecanii* (Schierling et al., submitted for publication). Compound **4** is limited to the *Stenus* species *S. solutus*, *S. cicindeloides* and *S. binotatus* (Lusebrink 2007). Concluding, the basal alkaloids **1** and **2** and the monoterpenoids **5**, **6** and **7** featuring the piperidine group are widely replaced by **3** and **4** in the epoxypiperidine group to achieve skimming ability and varied protection against harmful impacts. Summing up, it can be said that the secretion composition of *Stenus* is subject to an evolutionary optimization.

Due to its secretion composition consisting of **1**, **2**, **5** and **6** (Table 1), near to Steninae-related genus *Dianous* (Puthz 2001, 2008) represented in this study by *Dianous coeruleascens* can be regarded as phylogenetically basal like *S. comma* and *S. clavicornis* (Table 1). However, new investigations dealing with molecular analysis indicate genus *Dianous* to cluster in genus *Stenus* and not to be outlying (Koerner et al., personal communication).

Not only pure gland substances were analysed at the drop volume tensiometer but also naturally identical beetle secretions mixed according to quantification results. The spreading pressures of the naturally identical secretions of the beetle species *S. clavicornis*, *S. comma*, *S. similis* and *S. solutus* were determined (Table 5). For *S. clavicornis*, *S. comma* and *S. similis*, the spreading pressure values are almost the same. Only the spreading pressure value of *S. solutus* is minor due to the fact that the glands of this beetle species contain much of compound **4**. This compound is characterized by the lowest spreading pressure (Table 3) because it is factual not soluble in water. It possesses a resinous consistency and exerts no surface activity at all. It is imaginable that resinous **4** is dissolved by **3** in the glands of *S. solutus* beetles possessing **4** and therefore the beetles gain even though minor spreading potential. It is remarkable that generally there are no synergistic or quasisynergistic effects between the single compounds within the secretion as observed in rove beetles of Oxytelinae and Aleocharinae (Francke and Dettner 2005). Schierling et al. (submitted for publication) also reported no synergistic effects regarding the deterrent and antimicrobial function of the naturally identical *Stenus* secretion.

In conclusion, the *Stenus* secretion meets many different demands. The spreading potential of the gland compounds is not only essential as a locomotion or escape mechanism but also for the ability of the antimicrobial and protective

secretion to spread on body surface of the beetle while secretion grooming. While also defensive secretions of other insects serve as surfactants and are able to spread for example oral secretions regurgitated by *Spodoptera exigua* (Lepidoptera; Rostás and Blassmann 2009) and secretion of whirligig beetles (Coleoptera: Gyrimidae; Vulinec 1987), no other insect has developed striking skimming behaviour. Although extraordinary skimming is a secondary effect of the multifunctional *Stenus* secretion that primary was evolved for chemical defence, it is essential for the beetles' survival and helps *Stenus* and *Dianous* beetles to be best adapted to their varied habitats and challenges of life amongst all staphylinids.

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