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Sphaerium corneum and *Sphaerium nucleus* – sibling species or not?

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V Praze 28. 4. 2006 Kořínková

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Adam Petrusek

Svoluji se zapůjčením své práce pro studijní účely a prosím, aby byla vedena evidence výpůjček. Dále žádám, aby pramen převzatých údajů byl řádně citován.

CONTENTS

PŘEDMLUVA

Čeští malakozoologové v čele s legendárním Vojenem Ložkem se tradičně soustředili především na studium suchozemských měkkýšů. K většímu rozvoji poznatků o sladkovodních mlžích dochází u nás až v posledních 10 až 15 letech zejména zásluhou Luboše Berana a Michala Horsáka. V prvních fázích šlo o výzkum převážně faunistický, tj. shromažďování dat o rozšíření jednotlivých druhů, případně hledání spolehlivých znaků pro druhovou determinaci. V současnosti se pozornost obrací na detailnější studium jejich biologie, morfologie a anatomie.

Detailnější studium mlžů čeledi Sphaeriidae po stránce anatomické, konchologické a cytologické bylo inspirováno podobně zaměřenými zahraničními pracemi například Taehwana Lee (University of Michigan) a zejména předčasně zesnulého Alexeje Korniushina. Právě jím provedená revize zástupců čeledi Sphaeriidae v palearktické oblasti podnítila zájem o výskyt taxonu *Sphaerium nucleus* na území České Republiky. Po nalezení populací tohoto mlže na několika lokalitách se ukázalo potřebným znovu řešit otázku, zda se jedná skutečně o samostatný druh morfologicky podobný příbuznému druhu *Sphaerium corneum* či jen o jeho vyhraněnou ekomorfu.

Vzhledem ke zvolenému multidisciplinárnímu pojetí řešení tématu by bylo klasické uspořádání diplomové práce s jednotným úvodem, metodikou a výsledky nevhodné. Protože většina výsledků byla nebo v nejbližší době bude nabídnuta k publikování formou odborných článků, je celá práce členěna do oddílů odpovídajících jednotlivým článkům a psaných v převládajícím jazyce odborných publikací, tedy v jazyce anglickém. Tím se zároveň předešlo i určitým problémům s neexistencí zavedených českých termínů například pro některé anatomické struktury.

Ráda bych na tomto místě poděkovala všem, kteří se – ať radou, pomocí či technickou podporou, na vzniku této práce podíleli – jmenovala bych především svou školitelku Lucii Juřičkovou, Františka Šťáhlavského, laborantku katedry zoologie Miladu Řehákovou, profesora Jaroslava Smrže jakož i další kolegy z Oddělení zoologie bezobratlých, Jiřího Krále z Katedry mikrobiologie a genetiky, Adama Petruska z Katedry ekologie, Michala Horsáka z Masarykovy university v Brně, Luboše Berana (Správa CHKO Kokořínsko) a Vojena Ložka (Geologický ústav AV ČR). Můj dík patří také Danielovi Hradilákovi a Nicole Černohorské za jazykové korektury jednotlivých kapitol.

PREFACE

Species determination and taxonomy of molluscs has long been based prevailingly on shell characters. It is only in the last two or three decades when anatomy, life histories, cytogenetics and molecular biology are being more involved in molluscan studies. With the development of new methods, new problems emerged and extensive splitting occurred – many taxa, which would be considered uniform according to the "classical methods" proved to consist of separate, though closely related species (as far as water molluscs are concerned, e.g. "*Stagnicola palustris* agg." – now 5 species, or "*Radix peregra*" can be mentioned).

This is the case also with the family Sphaeriidae. Definitions of many species and subspecific forms as established by various authors were quite ambiguous. The still most successful attempt to make the taxonomy of this group more comprehensive comes from the eminent Ukrainian zoologist Korniushin, the author of over 20 articles dealing with revision of a set of anatomical and conchological characters and taxonomic status of the representatives of the family. His rather exact definitions and demarcations of taxa like *Pisidium globulare* (previously regarded as a form of *P. casertanum*), *Sphaerium ovale* (synonymised with *S. radiatum*) and *Sphaerium nucleus* (previously a form of *S. corneum*) persuaded many other malacologists to consider them as discrete species and to revise their occurrence in some european countries.

As the two supposedly sibling taxa *S. corneum* and *S. nucleus* appear on many sites in the Czech Republic, a complex analysis comprising both anatomical and morphometrical comparison in combination with histology, cytogenetics, molecular biology and evaluation of ecological preferences was considered very useful and advisable. In fact the aim of this study was twofold – primarily to highlight the relations between the chosen species, but at the same time to make a kind of model application of combined approach to taxonomic problems.

Combination of more methods in taxonomic studies has already been undertaken by some authors – e.g. Lee, who dealed with the entire family Sphaeriidae using morphological, cytogenetic and molecular data. Nevertheless, the choice of such a large group naturally leads to more superficial examination of many details of the anatomy. Also study of karyotypes of some species not inhabiting the respective country is hardly possible, as the methods usually require fresh material. Hence I choose for my diploma thesis a detailed and complex study of two taxa only, with the perspective to subsequently apply the approved methodics for other "problematic" taxonomic groups.

Because of the large amount of obtained data, the entire diploma thesis was from the beginning designed as a set of interrelated articles, prepared for publication.

CHAPTER 1

The first reliable records of *Sphaerium nucleus* (Mollusca: Bivalvia: Sphaeriidae) in the Czech Republic

(submitted to Acta Societatis Zoologicae Bohaemicae in July 2004)

The first reliable records of *Sphaerium nucleus* (Mollusca: Bivalvia: Sphaeriidae) in the Czech Republic

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Abstract. The first reliable records of *Sphaerium nucleus* (Studer, 1820) from the Czech Republic are presented. This species has been neglected for a long time, because of its conchological similarity to *Sphaerium corneum* (Linné, 1758).

Notes to the anatomy of both species are added.

Distribution, Anatomy, Mollusca, Bivalvia, Sphaeriidae, Palaearctic region:

INTRODUCTION

Sphaerium nucleus (Studer, 1820) has long been regarded as a subspecies or infrasubspecific unit of *Sphaerium corneum* (Linné, 1758), defined by conchological (more tumid shells with broad umbones) and ecological (preference for temporary habitats) characters. Strong evidence for the distinctness of *S. nucleus*, supported by some newly revised anatomical (type of kidney) and conchological (broad hinge plate, dense porosity) characters, is presented mainly by Korniushin (1994, 1996, 2001). Recently *S. nucleus* has been recognized as a distinct species by many authors, e.g., by Falkner (2000), who also indicates some peculiarities of its biology, namely a relatively long life span of 2-3 years and low juvenile mortality, both probably connected with living in an unpredictable environment.

Sphaerium nucleus or "*Sphaerium corneum* f. *nucleus*" has recently been reported from neighbouring countries (Falkner 2000, Mildner 2000, Piechocki 1989). Authors of the check-list (Falkner et al. 2001) even mention the Czech Republic among the countries with occurrence of this species. However, this was apparently done without revision of any material. The Czech authors referring to "*S. corneum* var. *nucleus*" (e.g., Uličný 1892, Ložek 1956) based their determination on only a few shell characters, where no attention was given to the anatomy. Therefore it is not possible to adapt these records as true *S. nucleus*. In the last 50 years distinction between the species (or subspecies according to the previous system) of the "*Sphaerium corneum* group" has been somewhat omitted and thus no record of *S. nucleus* in the Czech Republic/Czechoslovakia has been made (e.g., Beran 2002).

MATERIAL AND METHODS

In the summer of 2003 and 2004, localities in the Czech Republic with the typical habitats of *S. nucleus* (small more or less stagnant water bodies with dense vegetation) were visited. (The numbers of quadrates used in the faunistic grid map are according to the publication of Zelený 1972). The field collections were made using a bowl-shaped sieve. The living animals were observed for a short time (1-2 hrs), then killed and fixed in 70% ethanol or 3% formaldehyde (the specimens preserved in formaldehyde were not suitable for dissections). The dissections were carried out and both anatomical and conchological characters were observed under a stereomicroscope at a magnification 25 or 50 times. For examining the shell characters, the separate valves were put in 5% natrium hypochlorite (as recommended by Araujo & Korniushin 1999) to dissolve the organic layers.

COLLECTION SITES

(Fig.1)

(1) Water ditch (width at the collection site ca. 1 m, depth 0.5 m) with nearly stagnating water, muddy bottom and edges covered with water plants; approx. 2 km SE of Tvrdonice, S Moravia, CZ-7268, 48°44'58''; 17° 00'14'' [GPS]; 15 specimens examined

(2) Small drain (width 0.5 m, depth 0.5 m) with slowly running water and muddy bottom, partly covered with water plants; near the pond Kačák, Poodří Protected Landscape Area, ca. 1.5 km SE of Studénka, CZ-6274, 49°42'23''; 18° 05'15''[GPS]; 15 specimens examined

(3) Small pools (length and width of the largest one ca. 15 and 3m respectively, max. depth 0.7 m) with dense vegetation cover, Litovelské Pomoraví Protected Landscape Area, near the railway ca. 1.5 km E of Moravičany, CZ-6267, 49°45'04"'; 16°59'47"[GPS]; 2 specimens examined

RESULTS

Altogether 32 specimens were examined, and all of them possessed the main characters of *Sphaerium nucleus* (according to Korniushin 1994, 2000): comparatively small shells, nearly oval in outline, with broad umbones and hinge plate, scars of upper siphonal retractors separated from those of posterior adductors, pores in the shell apparently more numerous (although a statistical comparison was impossible due to lack of material) and more evenly distributed than in *S. corneum*, dorsal lobe of nephridium of the closed type. Of the other anatomical structures, attention was paid mainly to the arrangement of the alimentary tract, siphonal muscles and brood pouches. The alimentary tract is not markedly different from that of *S. corneum* (the main characters are the same as those indicated in the literature, e.g., Korniushin & Glaubrecht 2001) – the stomach is stretched in the posterior direction, its transition into the midgut is not marked, major typhlosole (t1) forms two loops in the stomach, minor typhlosole (t2) runs towards the relatively narrow sorting area. The intestine forms a complicated coil with 2 loops. The number of brood pouches per 1 demibranch is up to 5, each containing usually 2-3 embryos or larvae. As in the case of porosity, more material needs to be examined in order to evaluate the intra- and interspecific variability in this character.

(Figs. 2-3)

DISCUSSION

This is the first time *Sphaerium nucleus* is being reported from the Czech Republic as a distinct species and its main characters, previously stated as constant throughout the

Palaearctic region, proved to apply to the populations from this country. However, in certain types of biotopes this species could be much more common. There is thus a need to examine all material from swamps, bogs, ditches and other temporary habitats more thoroughly and, possibly, revise older museum collections.

It is also remarkable that both in the Poodří Protected Landscape Area and Tvrdonice *S. nucleus* is found in localities not far from these in which *Anisus vorticulus* (Troschel 1834) (Horsák 2000, Beran & Horsák 1998), *Pisidium pseudosphaerium* (Favre 1927) and *Pisidium globulare* (Clessin 1873) (Horsák & Neumannová 2004) were reported. The occurrence of *A. vorticulus* and *S. nucleus* in the same habitats has already been noticed by Falkner (2000). Therefore, the occurrence of those species in the same malacocenoses can be assumed to be a common phenomenon.

Acknowledgements

I would like to thank Michal Horsák (Masaryk University in Brno) for information on localities, Alexei V. Korniushin (Ukraian Academy of Sciences in Kiev) for advice on the determination of the material, Lucie Juřičková (Charles University in Prague) for critical revision of the manuscript and Daniel Hradilák for language revision.

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FIGURES

Legend to the figures

Fig. 1 – Localities where *S. nucleus* was found

Fig. 2a – *S. nucleus* - shell in lateral view, Fig. 2b – scars of posterior adductor and siphonal retractor Fig. 2c – detail of hinge plate from left valve, Fig. 2d – detail of hinge plate from right valve,

u – umbo, c2, c3,c4 – cardinal teeth, a1, a2, a3 – anterior lateral teeth, p1, p2, p3 – posterior lateral teeth, p.a. – scar of posterior adductor, s. r. – scar of siphonal retractor

Figs. 3a, 3b – dorsal lobe of nephridium - *S. nucleus* (closed type of nephridium, Fig. 3a), *S. corneum* (open type, Fig. 3b).

dl – dorsal lobe, pt – pericardial part of the nephridial tubule, pa – posterior adductor

Fig. 3c – *S. nucleus* -part of alimentaty tract in lateral view (coil of intestine not in its natural position).

Fig. 3d – *S. nucleus* -inner structure of the stomach

oes – oesophagus, st – stomach, md – midgut, af – anterior fold, sa – sorting area, t1 – major (anterior) typhlosole, int – intestine

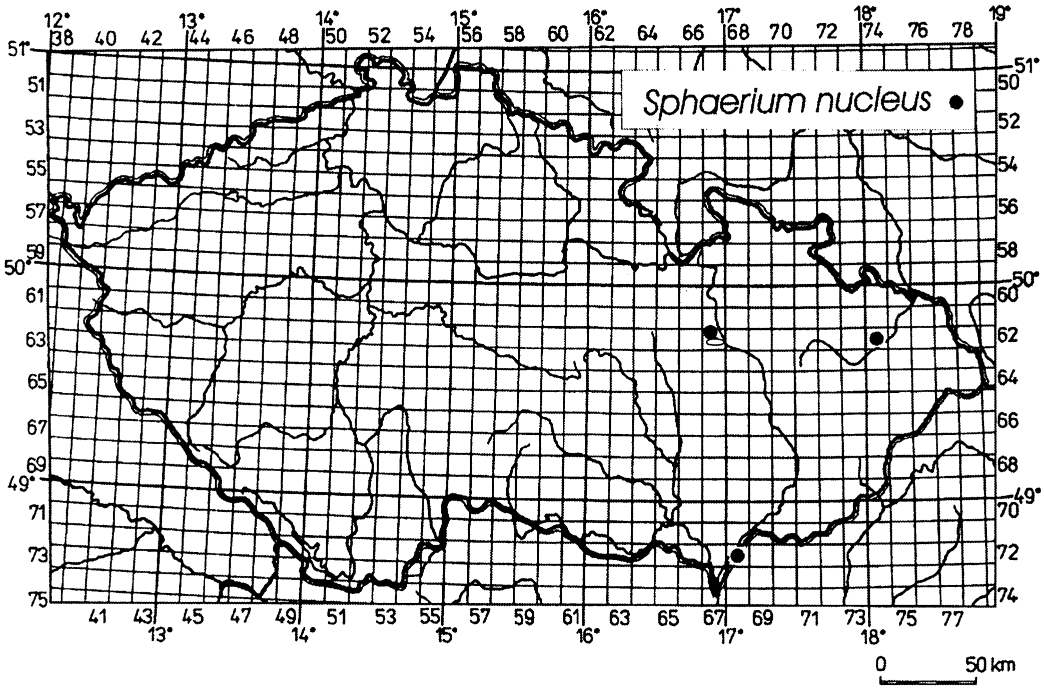

Fig.1

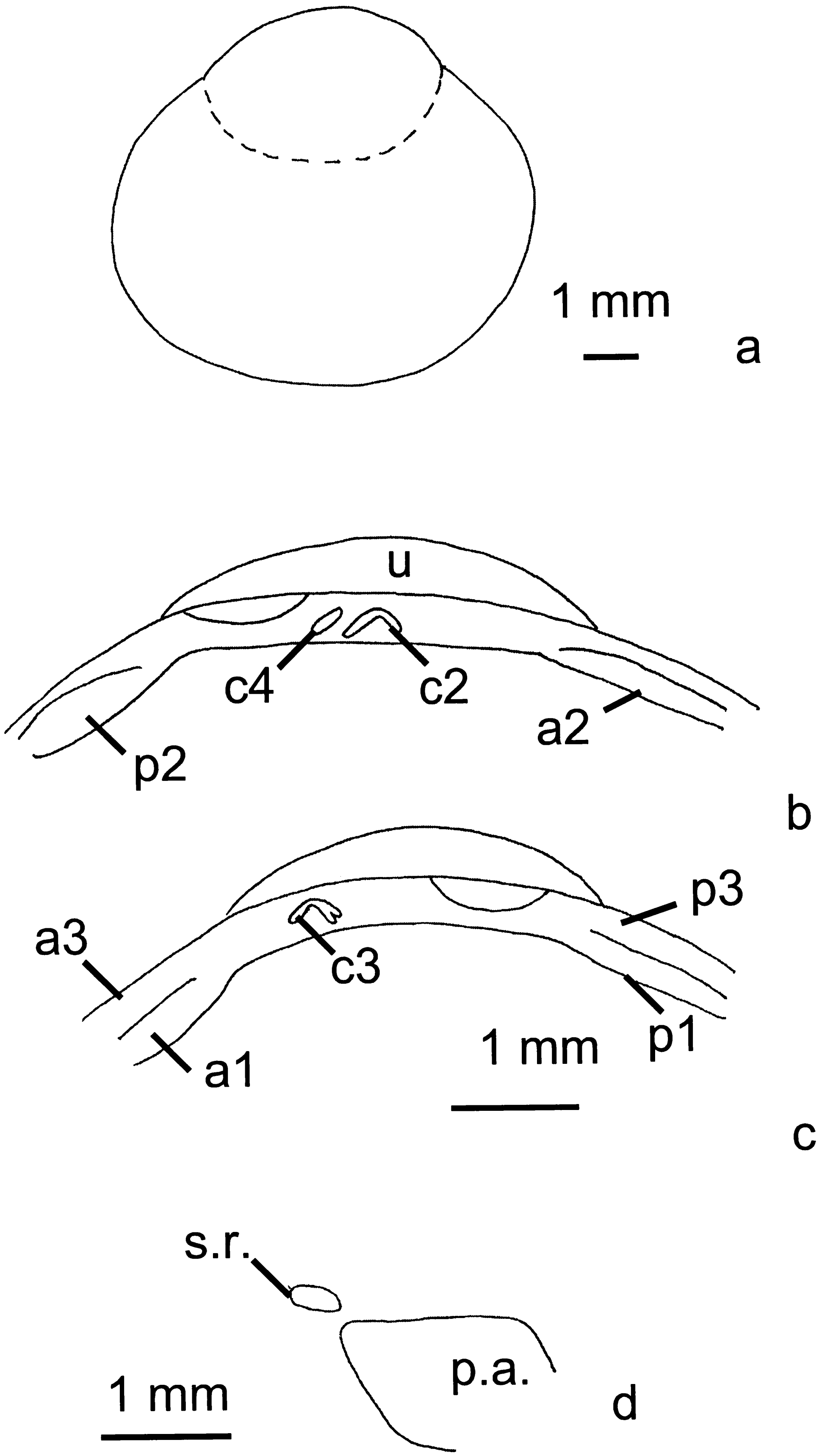

Fig. 2

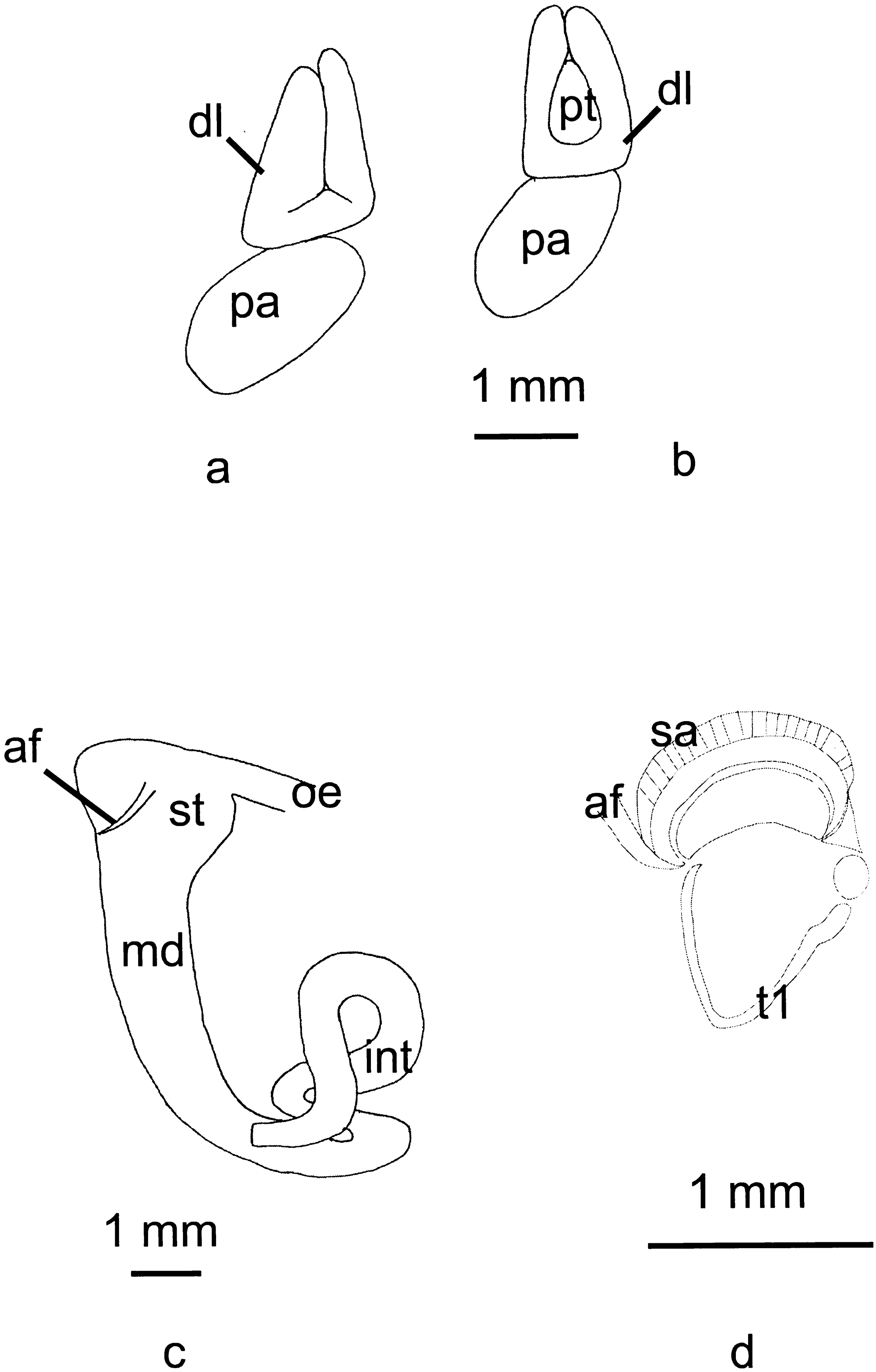

Fig. 3

CHAPTER 2

Revision of the anatomical, conchological and ecological characters

(Prepared for publication in Journal of Conchology)

SOME ANATOMICAL, CONCHOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF SIBLING TAXA *SPHAERIUM CORNEUM* AND *SPHAERIUM NUCLEUS*

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Abstract

This study compares specimens of 8 populations of S. corneum and 6 populations of S. nucleus sampled from the Czech Republic, accentuating differences in shell porosity, microanatomy of the mantle, shape of kidneys and arrangement of the brood pouches. Most of the observed characters are assumed to be connected with the characteristics of habitat.

Key words: Sphaerium, shell porosity, anatomy

INTRODUCTION

The taxonomic rank of *Sphaerium nucleus* and *Sphaerium corneum* remains doubtful. In preceding decades, the former taxon has been prevailingly placed as a form or subspecies of the latter. Since Korniushin´s taxonomic revision (1994, 2001), the discreteness of *S. nucleus* was commonly accepted and supported by some new evidences - (Falkner 2000, Chernyshova & Kiritchuk 1998.).

In principle there are two attitudes to the evaluation of shell morphology: the landmark-based one, focused on linear measurements (quotients comprising shell length, height, width, length of the hinge plate etc.), and the outline-based one, which takes use of image analysis. The application of both methods to *Sphaerium* is dealt with by Kořínková, Petrusek and Juřičková (in prep.), who succeeded in evaluation of the differences in the outline using image analysis and elliptic Fourier transformation. As discussed by these authors and by Korniushin (1994, 2001), combinations of linear measurements (which are the easiest to obtain) fail to distinguish between the two taxa reliably, and thus the use of other characters (shell porosity, anatomy of soft parts) is needed for determination.

However, even if reliable characters for determination are set, there still remains the problem of different concepts of species – mainly of the definition of morphological, ecological and biological species. Differences in conchological characters or ecological requirements do not by itself mean an existence of reproduction barrier, as does e.g. the pronounced difference in karyotypes.

This study does not claim to bring a definite solution of the problem above mentioned, but rather to evaluate variability in some of the characters.

MATERIAL AND METHODS

MATERIAL SAMPLING AND ANATOMICAL EXAMINATION

From summer 2003 to autumn 2005, both living specimens and empty shells were sampled from sites in the Czech Republic (see the list of collection sites in the Appendix). Collections were made using a bowl-shaped sieve. Specimens were conserved in 70% ethyl alcohol and dissected and measured under a stereomicroscope (magnification up to 50 times). The following anatomical characters were noticed and evaluated: shape of kidney, number of larvae per brood pouch and percentage of retarded larvae. Larvae at lower developmental stage than other animals in the same brood (according to Hetzel 1993) or attaining less than 50% (arbitrarily chosen limit) of the shell length of their kin in the brood (i.e. enclosed in the same brood pouch) were considered as retarded.

EXAMINATION OF SHELL CHARACTERS

On the larger shells (length >4 mm), three major parameters (shell length – L, width – W, height – H) were measured using slide calliper to the nearest 0.1 mm. For the study of pores, shells were placed into 5% sodium hypochlorite for 24 hrs. in order to remove the organic layer, then rinsed in distilled water, air-dried and observed under stereomicroscope. Number of pores was counted on a square area 0.2x0.2 mm always in the part with the highest density of pores near the umbones (but not in the most curved part).

HISTOLOGICAL SECTIONS

After being killed, most animals were preserved in 70% ethyl alcohol for a few days and their shells removed by hand. Animals with thin and fragile shells were placed in Bouin´s fixative for 24-48 hours in order to remove the calcium salts, and then again in 70% ethyl alcohol to remove the remains of picric acid.

In both cases, the soft tissues were then dehydrated by successive immersing in 96% ethyl alcohol (6-12 hrs. according to the size of the animal), propyl alcohol (2x 6-12 hrs.), propyl alcohol-methylbenzoate (3-6 hrs.), methylbenzoate (6-12 hrs.), benzene (12-24 hrs.), benzene-paraffin (12-24 hrs.) and finally paraffin. After stiffening, the blocks of paraffin were sectioned at 1-10 (usually 5 μm) using Leica- microtome, stained (after removal of the paraffin using xylene and series of 96%-80%-60%-40% ethyl alcohol) in Masson´s triple stain and, after dehydratation in propane and xylene, fitted in Canada Balsam.

MEASURING ENVIRONMENTAL VARIABLES

On 10 selected sites (underlined in the list in Appendix), temperature, pH, conductivity and dissolved oxygen were measured with YSI 556 MPS multiprobe (YELLOWSPRINGS INSTRUMENT). Measurements were carried out in autumn 2004 and spring 2005 to compare the values of all respective variables in two different periods of the year.

RESULTS

Habitat preferences

Box plots (Fig 3, 4) illustrate the range of pH values (from 3.8 to 9) and dissolved oxygen (from 0.2 to 150% of total saturation) on 10 chosen collection sites. It is evident that *S. nucleus* can tolerate extremely low oxygen levels (less than 1% of total saturation) during some periods of the year (the difference in the oxygen content in autumn, after the summer stagnation, is highly significant – $p=0.0048$, for measurements from spring $p=0.31$, for both spring and summer $p=0.036$ – results of t-test for independent samples, grouping variable=species).

S. corneum is considerably euryvalent (the list of collection sites includes both small brooks, large rivers, as well as races with gentle water and silt on the bottom), but likely to avoid extremely unstable and temporarily anoxic habitats typical for *S. nucleus*. No locality with simultaneous occurrence of the both has been found so far (Kořínková 2006, Korniushin 1996, 2001, Mildner 2001, Falkner 2000, present study).

Shell and shell porosity

All representatives of *S. nucleus* revised for this study could be characterized by broad-oval outline of valves (Fig. 2), without pronounced angles, whereas the shells of some populations of *S. corneum* exhibit stronger variability of shapes – from oval (often undistinguishable from *S. nucleus* by naked eye) to nearly trapezoid (Fig.1). The specimens of *S. nucleus* can be generally assigned as more tumid (having larger shell width than representatives of *S. corneum* attaining the same shell length), which can be demonstrated by higher values of constants in the formulae of allometric growth (see Kořínková, Petrusek, Juřičková in prep.).

The colour of periostracum varies a lot both with environment and with age of the animal – the younger specimens are usually yellow to brownish, and unicolour, adults of *S. nucleus* bear nearly always darker stripes concentric with the umbones.

As shown in the box plot (Fig 5), there is significant difference ($p<0.00$) in the pore density – the number of pores at 0.2x0.2 mm ranges from 12 to 45 in shells of *S. nucleus* (average 28.9±8..65, median 30), whereas in *S. corneum* it is from 7 to 26 only (average 14.5±4.05,. median 13.5). The correlation between pore density and shell length is positive in *S. nucleus* and negative in all but one populations of *S. corneum*.

As illustrated by Fig. 5, there is strong ($r=0.87$) and negative correlation between pore density and oxygen content in the autumn.

ANATOMY OF SOFT PARTS

Both the species are much alike in most of the anatomical characters and follow the general anatomical pattern of the most of the Sphaeriidae (see e.g. Monk 1931). On histological sections, differences can be found in the density and length of lateral projections of "caecal cells" (more than 100µm in *S. nucleus*, about 50 µm in *S. corneum*) present predominantly in the outer layer of mantle epithelium.

The shape of the kidney is presented as one of the most important diagnostic characters by Korniushin, who describes *S. corneum* as having an "open type" of nephridia (i.e. with the pericardial part of tubule visible between the branches of the dorsal lobe), whereas *S. nucleus* is asserted to have nephridium of broadened, almost square shape and "closed type" (the branches of dorsal lobe lying close to each other). Although this condition could be observed in the majority of Czech material, some irregularities took place. In some populations of *S. nucleus*, up to 15% of specimens had at least one nephridium of the pair with a more or less distinct gap between the branches of the dorsal lobe, whereas the outline of the dorsal lobe remains broadened in all cases (see Table 1). And reversely, in some populations, which were determined as *S. corneum* according to other characters, gradual transitions between the "open" and "closed" type were observed (see Fig 9).

The number of embryos or larvae per brood pouch, including retarded larvae, ranged up to 11 in populations of *S. corneum* inhabiting larger rivers, but never exceeded 8 in representatives of *S. nucleus* (see Table 2).

DISCUSSION

The measurements of environmental variables are fully in agreement with Korniushin (1996, 2001), Mildner (2001) and Falkner (2000), who reported *S. nucleus* as typical for temporary drains, pools and swamps with dense vegetation cover and low content of dissolved oxygen.

Pore density is regarded as an important taxonomical character (Dudych-Falniowska 1988, Kiritshuk and Stadnichenko1998) and has already been used as a character for distinction between morphologically similar taxa, e.g. *Pisidium casertanum* and *P. globulare* (Araujo & Korniushin 1998, Horsák & Neumannová 2003). This study is presumably the first to bring concrete numeric data about porosity in the case *S. nucleus* vs. *S.corneum*. The results correspond to the assertions of Korniushin that the former has more numerous and more evenly distributed pores than the latter.

Results obtained by the method of histological sections confirm the finding of Chernyshova and Kiritshuk (1998), that *S. nucleus* bears longer lateral projections of caecal cells than does *S. corneum*. This together with the above mentioned findings could lead to the assumption that porosity and number and length of caecal cells are in some relation and also hang together with the environment.

The same case is probably with the number of larvae per brood pouch and larval retardation. Although the differences are not statistically significant ($p=0.71$), the prevailingly lower number of larvae (as already observed by Falkner 2000) and higher ratio of retardation in populations of *S. nucleus* would agree with the assumption made by Hetzel (1993), that retardation represents an adaptation to unstable habitat.

Thus, the present study shows outstanding differences between the taxa *S. corneum* and *S. nucleus*, independently of the rank, which they are just now ascribed to (independently of whether they are regarded as separate species, or ecological forms).

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Table 1– open and closed types of nephridia

Species	population	total number of specimens	n. spec. with closed nephridia	n. spec. with open nephridia
S. corneum	Alba	25	0.5	24,5
	Bechyn	25	3	22
	Cidlina	20	0	20
	Dírenský	20	1	19
	Vltava	19	0	19
	Maškův	25	25	0
	Rokytka	20	0	20
	Sánský	20	2	18
	Vidnava	20	0	20
S. nucleus	Kačení louka	2	2	0
	Kotvice	3	3	0
	Libice	15	13	2
	Popovice	10	8	2
	Tvrdonice	15	14.5	0.5
	Žabakor	5	3	2

Table 2 – number of larvae per brood pouch and larval retardation

Species	population	total n. of specimens	n.. of larvae per brood pouch		% of retarded larvae
			range	average±s.e.	
S. corneum	Alba	25	2-9	3.61±1,57	3.2
	Bechyn	25	1-12	5.53±2.,21	3.5
	Cidlina	20	1-10	3.88±-1.74	4.7
	Dírenský	20	1-10	1.28±-1.58	5.8
	Vltava	19	1-11	4.85±-2.62	5.6
	Maškův	25	1-7	2.25±1.06	3.23
	Rokytka	20	1-7	2.76±1.15	9.9
	Sánský	20	2-10	4.,8±1.9	2.1
	Vidnava	20	1-2	1.35±0.,48	1.1
S. nucleus	Kačení louka	2	1-7	1.5±0.51	1.5
	Kotvice	3	1-7	3.06±1.29	7.1
	Libice	15	1-6	3.1±1.03	5.3
	Popovice	10	1-8	3.82±2.25	10.8
	Tvrdonice	15	1-5	2.55± 0.9	<1
	Žabakor	5	1-6	2.94±1.22	11.9

APPENDICES

LIST OF COLLECTION SITES

1. Alba 50°09′09′′ ;16°05′08′′ CZ-5862a-b- the Alba race N of Týniště nad Orlicí, running water, bottom: sand, mud, leaves and other organic material, width ca. 2 m, depth at collection site ca. 40 cm.. *Sphaerium corneum*, high abundance (10-20/m^2) (25 specimens dissected, 20 shells examined), T. Kořínková; 2. Bechyňský potok 49°14′0‘‘; 14°39′20′′ CZ-6753d- Bechyňský potok near the road bridge, ca. 2 km S of

Záluží, running water, coarse-grained (particles ca. 2 mm in diameter) sand and partly silt. width ca. 150 cm, depth 20 cm

Sphaerium corneum high abundance (over 20/m^2) (25 specimens dissected, 13 shells examined); 3. Cidlina 50°7′19′′; 15°10′3′′ CZ-5857c – river Cidlina ca 2 km SW of Libice nad Cidlinou, near the bridge, bottom: presumably sand, width ca. 10m, depth at collection site 0,7 m .*Sphaerium corneum* (low abundance, <5/ m^2) (20 specimens dissected, 16 shells examined); 4. Dírenský potok 49°14′56′; 14°45′21′′ CZ-6754d - Dírenský potok near the bridge in the village Přehořov, running water, bottom: stones, sand, width ca 5 m, depth 30 cm. *Sphaerium corneum* (low abundance, <5/ m^2, restricted to sand between blocks) (20 specimen dissected); 5. Kačení louka 49°45'04'''; 16°59'47'' CZ-6267, small pools (length and width of the largest one ca. 15 and 3m respectively, max. depth 0.7 m), dried/out during summer, with dense vegetation cover, Litovelské Pomoraví Protected Landscape Area, near the railway ca. 1.5 km E of Moravičany, bottom: silt, organic material. *Sphaerium nucleus* (<1/ m^2) (2 specimen dissected, 9 shells examined); 7. Kotvice 49°42'23''; 18° 05'15'' CZ-6274, Small drain (width 0.5 m, depth 0.5 m) with slowly running water and muddy bottom, dried/out during summer months, partly covered with water plants; near the pond Kačák, Poodří Protected Landscape Area, ca. 1.5 km SE of Studénka. *Sphaerium nucleus* (high abundance (>100/ m^2), but burrowed deep into sediment in some periods of year) (15 specimens dissected, 17 shells examined); 8. Libický luh 50°6′42′′ 15°9′45′′ litoral of a pool in a flood-plain forest near Libice nad Cidlinou, depth at collection site 10-20 cm. *S. nucleus* (15 specimen dissected), collected by L. Beran; 9. Maškův mlýn 49°59′55′′; 14°19′05′′ CZ-6051a-b, old mill-race connected to Radotínský brook, ca. 2 km SW of Praha-Radotín, Český kras Protected Landscape Area, stagnant or running water depending on the season, bottom: silt, leaves and organic material, width 1 m, max. depth 0, 5 m. *Sphaerium corneum* (high abundance > 100/ m^2) (25 specimen dissected, 18 shells examined); 10. Popovice 50°15′25′′ 15°40′46′′ CZ-5760a, 2 small (ca. 3 m in diameter) pools near Bystřice river, on the verge of village Popovice, stagnant water (site dry on the date of collection) with dense vegetation cover, reed, bottom: silt and organic material. *Sphaerium nucleus* (>10/ m^2) (10 specimens dissected, 10 shells examined); 11.Rokytka 50°04′43′′ 14°36′48′′ CZ-5953a-b, brook Rokytka close to the built-up area of Praha-Běchovice, running water, bottom: silt, gravel and stones, width ca. 2 m, max. depth 0, 7 m. *Sphaerium corneum* (irregular distribution, somewhere > 50/ m^2) (27 specimens dissected, 24 shells examined); 12. Sánský kanál 50°11′24′′ 15°05′38′′ CZ-5856a-b- Sánský kanál between Křečkov and Budiměř, running water, bottom: sand, mud, ca. 10 cm layer of leaves, small picks and other org. Material, width ca 150 cm, depth at collection site 30 cm. *Sphaerium corneum* (irregular distribution, somewhere > 30/ m^2) (20 specimens dissected, 20 shells examined); 13.Tvrdonice 48°44'58''; 17° 00'14'', CZ-7268 Water ditch (width at the collection site ca. 1 m, depth 0.5 m) with nearly stagnating water, muddy bottom and edges covered with water plants; approx. 2 km SE of Tvrdonice, S Moravia, *S. nucleus* (irregular distribution, somewhere > 20/ m^2) (15 specimens dissected, 20 shells examined); 14. Vltava 50°05′37′′ 14°25′02′′ CZ-5952a-b, littoral zone of the Vltava river in Prague, near Čechův most (road bridge), bottom: sand, depth at collection site ca. 1 m. *Sphaerium corneum* (12+5 specimens dissected); 15. Vidnava 50°22′59′′ 17°12′00′′ CZ-5669a water running from the wetland ca. 0,5 km N of Vidnava, max. width 1 m, depth 0, 5 m. *S. corneum* (high abundance > 50/ m^2) (20 specimens dissected); 16. Žabakor 50°33′0′′ 15°3′3′′ CZ-5456d - litoral zone of a pond. S. nucleus (5 specimens dissected), col. by L. Beran

FIGURES

Fig. 1: *Sphaerium corneum* – from Vltava (left – trapezoid shape with pronounced angle, shell length 11.5 mm, left valve) and Alba (right – oval without angles, shell length 9 mm)

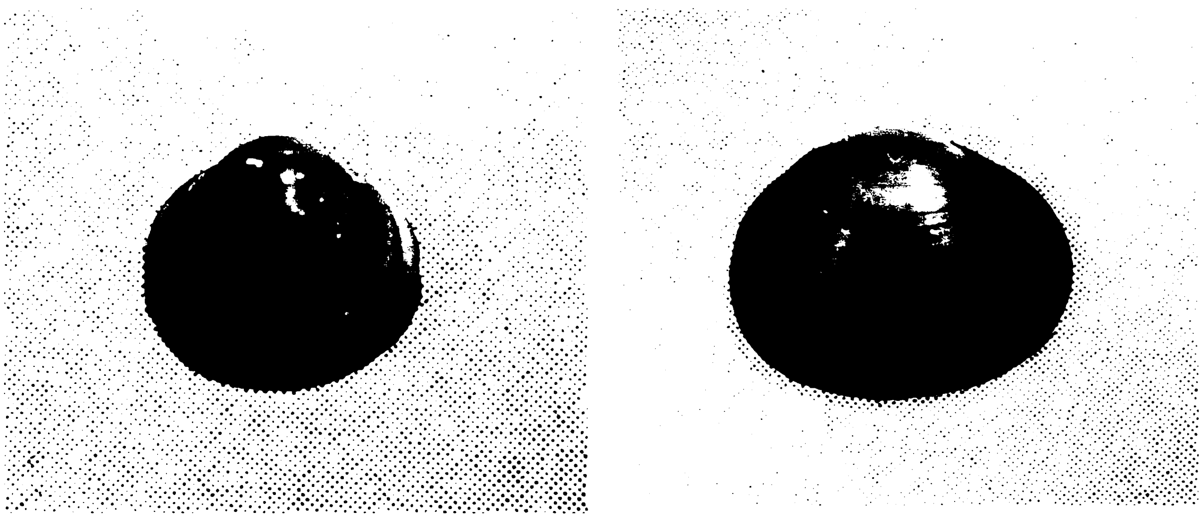

Fig. 2: *Sphaerium nucleus* from Popovice (on left; shell length 6.5 mm, right valve) and Tvrdonice (on right; shell length 8.5 mm; rigth valve)

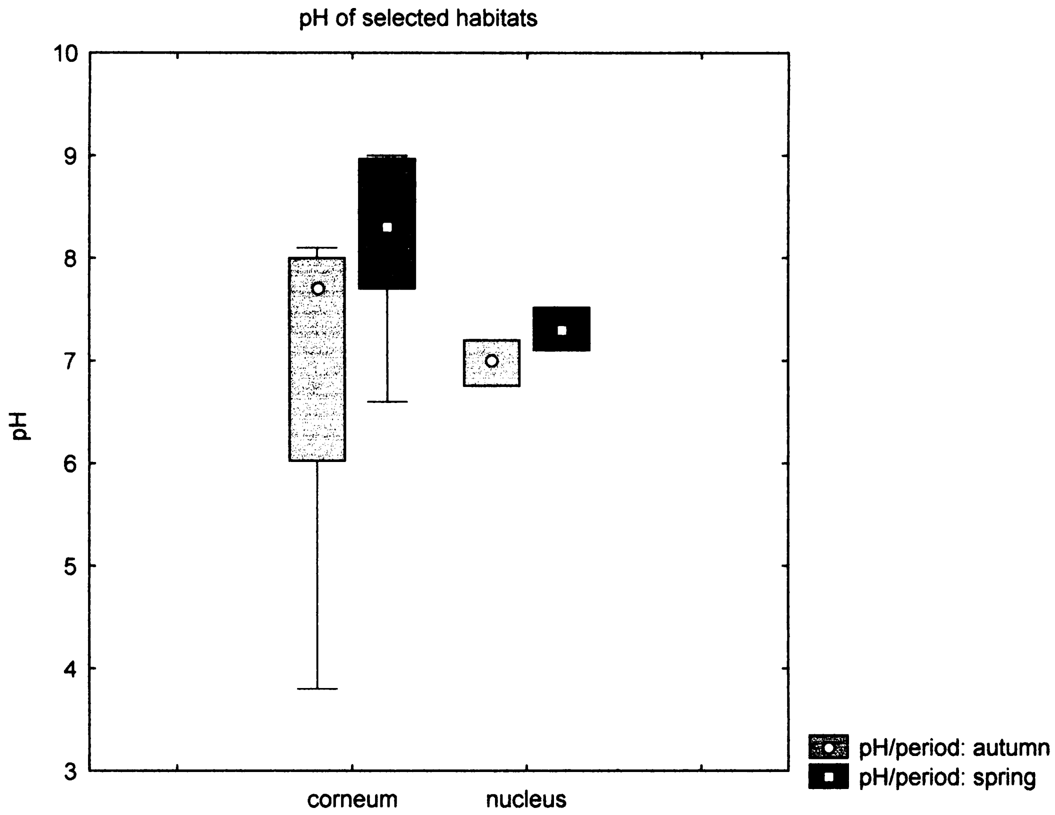

Fig. 3: Box plots illustrating ranges of pH values on selected sites of *S. corneum* and *S. nucleus*

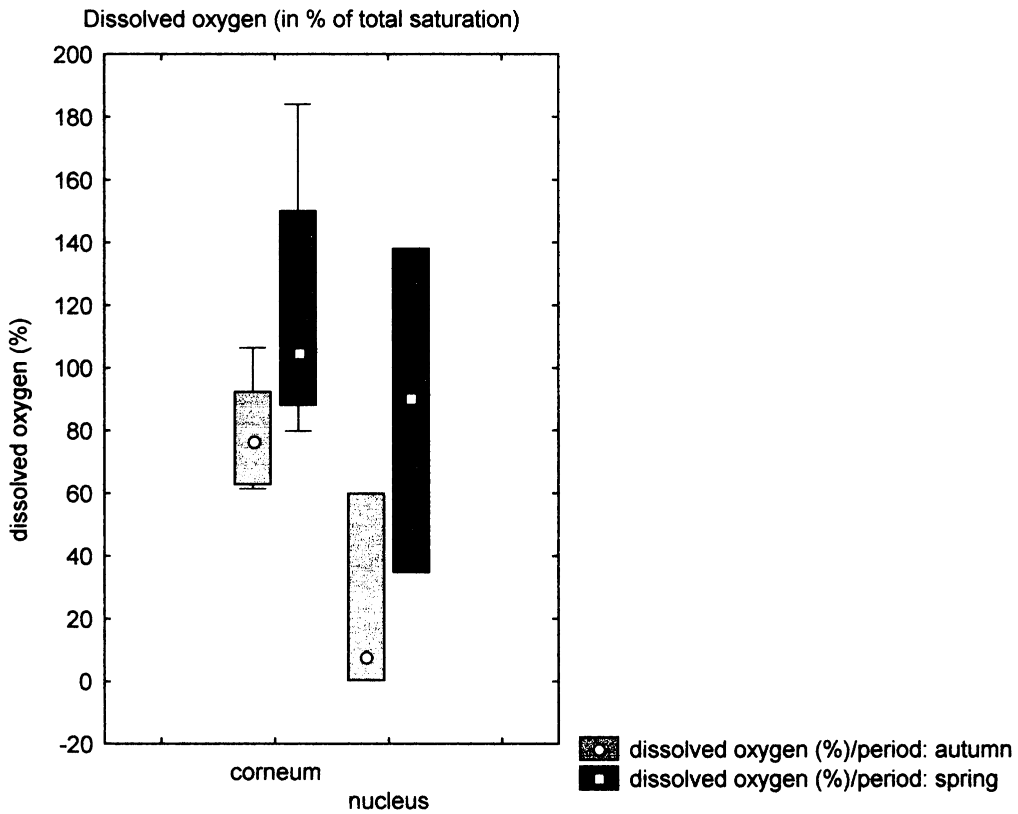

Fig. 4: Box plots illustrating oxygen content on selected sites of *S. corneum* and *S. nucleus*

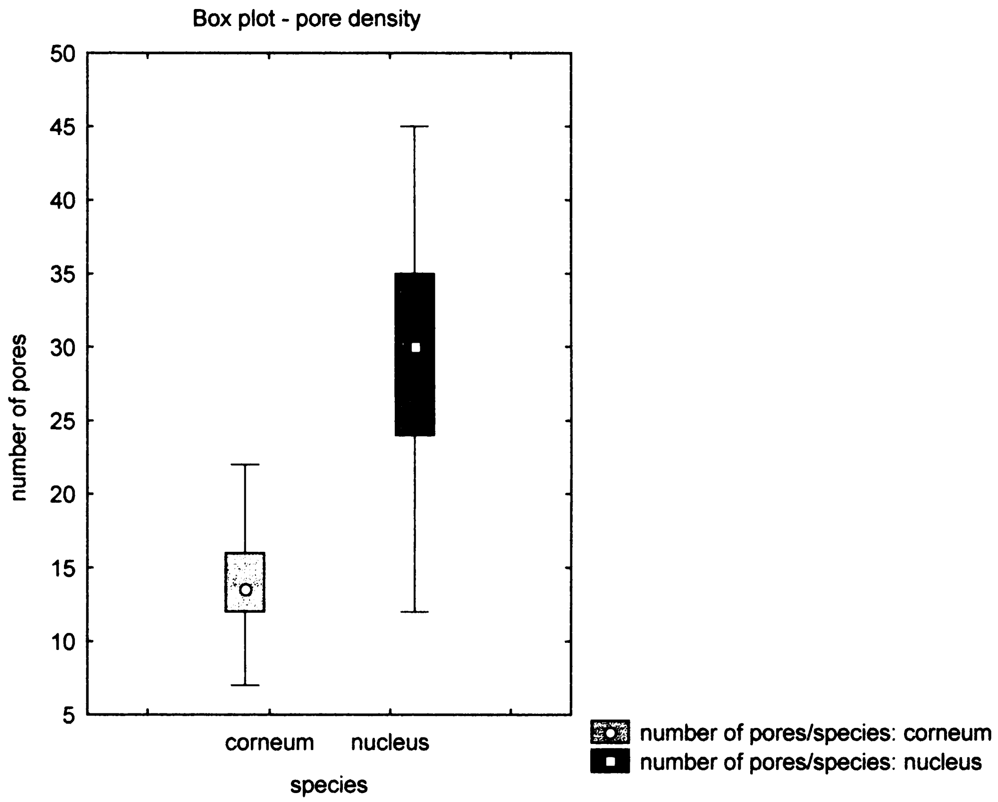

Fig. 5: Comparison of pore density (number of pores on the area of 0.04mm^2) in the shells of *S. corneum* (number of specimens=127) and *S. nucleus* (number of specimens=47)

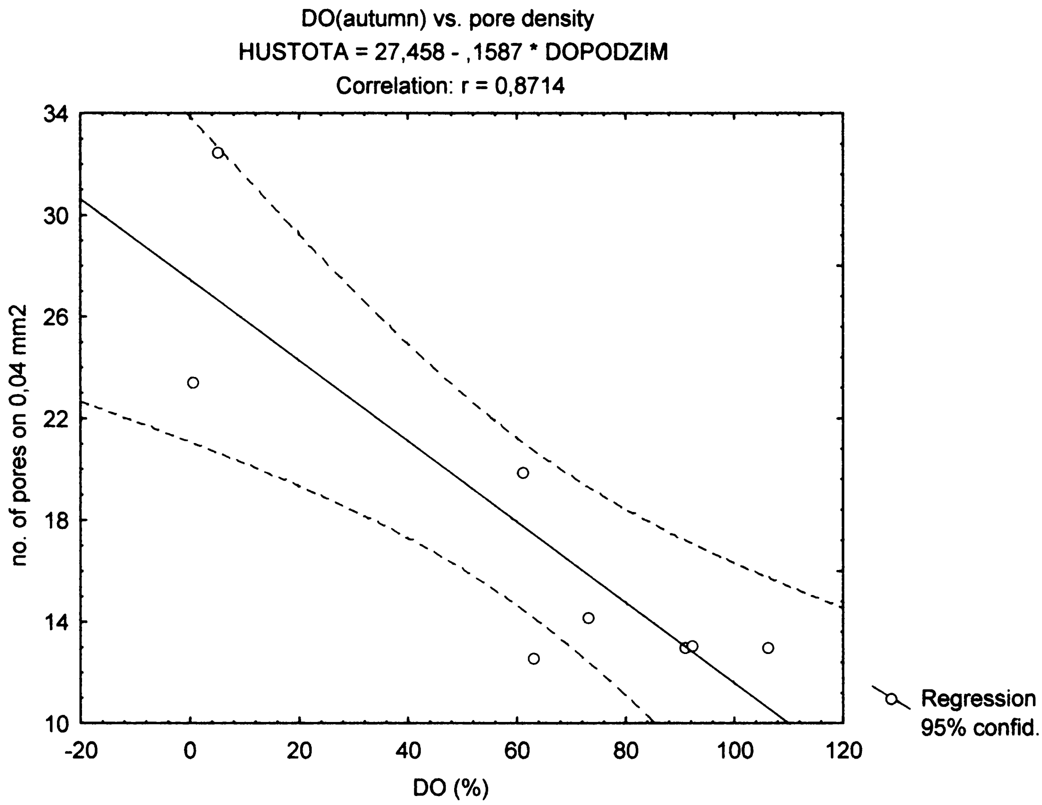

Fig 6: Correlation between pore density and dissolved oxygen in the autumn

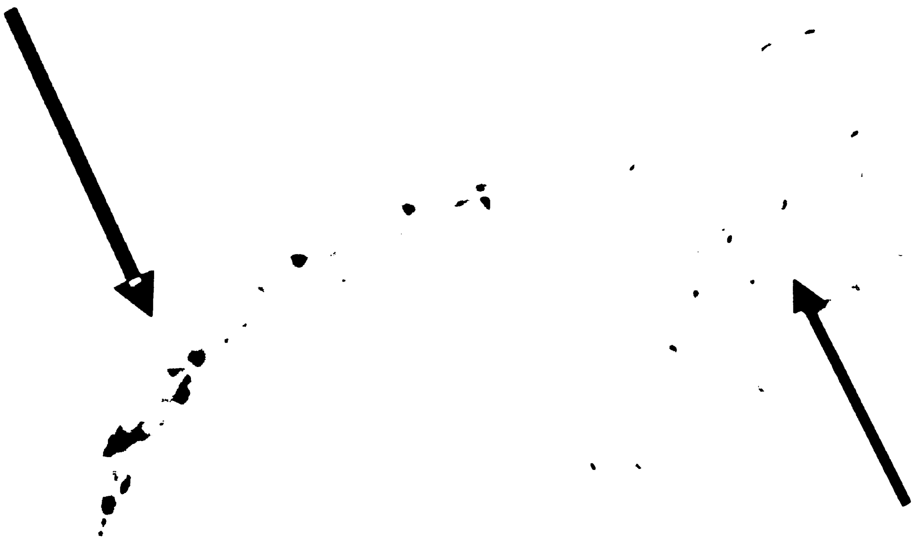

Fig. 7: Caecal cells (red arrow) of the outer layer of mantle epithelium of *S. corneum* (left) and *S. nucleus* (right). Scale bar 0.1 mm

Fig. 8: Typical habitats of *Sphaerium nucleus* – small temporary pool near Moravičany (left) and water ditch near Tvrdonice (right)

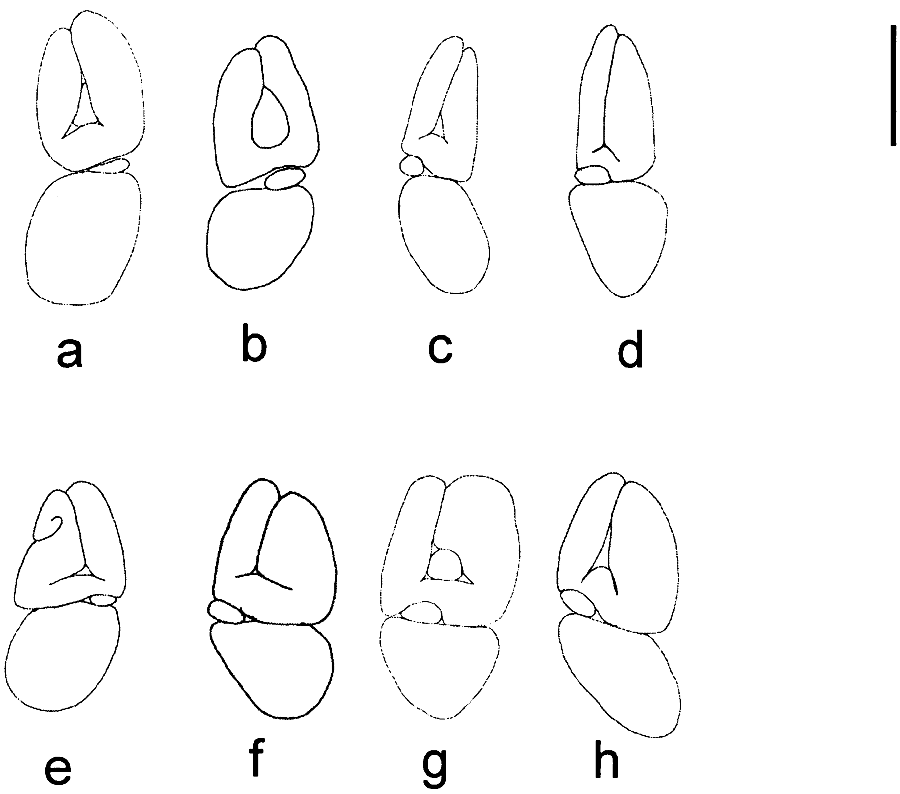

Fig. 9 : Nephridia of *Sphaerium corneum* (a, b – Maškův mlýn, left nephridium typical open type, c, d – Dírenský potok, right nephridium) and *Sphaerium nucleus* (e – Popovice, left nephridium, f – Tvrdonice, right nephridium – typical shape; g – Popovice, h - Libice – right nephridium, atypical shape)

Scale bar 1 mm

CHAPTER 3

Shell morphometrics
(prepared for publication in Journal of Conchology)

COMPARISON OF ELLIPTIC FOURIER TRANSFORMATION AND LANDMARK-BASED MORPHOMETRICS IN DISCRIMINATION BETWEEN THE CRYPTIC SPECIES *SPHAERIUM CORNEUM* AND *SPHAERIUM NUCLEUS* (BIVALVIA: SPHAERIIDAE)

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INTRODUCTION

Descriptions of species were traditionally based on morphological characters, and thus many questionable subspecies and forms of uncertain rank can be found in any taxonomic group. Detailed morphometric analysis, alone or in combination with other (often molecular -see e. g. Pfenninger et. al. 2003) methods, has many times approved itself in discrimination between hardly distinguishable species (Cale et. al. 2002) or, conversely, showed description of some supposed species or subspecies to be based on intraspecific phenotypic plasticity (Babik & Rafiński 2000).

Thank to the relatively simple shape of their shells, Bivalvia represent ideal objects for both landmark-based and outline-based morphometrics.

Landmark-based morphometrics have been applied to the research of this group for over 100 years. Usually three main pairs of landmarks were distinguished, the distances between them corresponding to the three main dimensions: the length (L), the height (H) and the width (W, also referred to as diameter – D by some authors).

To compare the shapes among individuals of different shell length, various indices of proportions were used – e.g. the height index or "relative height" (Korniushin 1994) H/L, the roundness index (Holopainen & Kuiper) or "relative thickness" (Korniushin 1994) D/H, the "obesity" W/L. However, the use of linear ratios is unadvisable for those groups, which show allometric growth, i.e. the relation between two measurements (e.g. height vs. length, width vs. length) can be expressed by logarithmic equation (Alimov 1967). This is the case also with Bivalves - the values of the indices can vary significantly among length classes in one population (as shown e.g. by Graf 1997). The above-mentioned nonlinearity is probably the reason of failure of some attempts to evaluate the morphological variation (Hornbach et al. 1980, Bailey et al. 1983). Also the factor analyses performed by Korniushin (1994, 1996, 2001) revealed significant individual variation of landmark-based metric characters and therefore failed to provide good diagnostic features for the species identification.

In the last two decades, outline-based methods have become more widespread. Both eigenshape analysis (e.g. Ubukata 2003, Guralnick 2005) and elliptic Fourier analysis (Lockwood 2004, Harries et al. 1996) have been used to describe shells of both recent and fossil bivalves.

Elliptic Fourier analysis (EFA) expresses the image outline (i.e. a set of x/y coordinates) as a summation of a certain number of harmonic functions. The higher number of functions, the more precise description of the outline curve is obtained. An apparent advantage of this method is the possibility to use the elliptic Fourier coefficients as data for multivariate statistical methods.

In this study, we applied the combination of EFA with principial component analysis (PCA) and permutation tests to the problem of two closely related and hardly distinguishable taxonomic units, *Sphaerium corneum* and *Sphaerium nucleus*. The taxonomical rank of the both remains doubtful. For this study, we decided to accept them as separate species but in past these taxa were often regarded only as conchological forms – *S. corneum* f. *corneum* and *S. corneum* f. *nucleus* (or „var. nucleus"), with quite vaguely defined differences. The most comprehensive, most exact and commonly accepted definition of S. nucleus as a species comes from Korniushin (1994, 1996), who stated both anatomical (the shape of kidney), ecological (prevalent occurrence in temporary habitats) and conchological characters. He describes the shell of S. nucleus as broad-oval in outline and without angles (which are often present in *S. corneum*), comparatively more thick-walled than in the latter species, and with maximum convexity in the midportion (while the shell of *S. corneum* has the maximum convexity in the upper portion and the frontal section is almost heart-shaped). Thus the main conchological difference between these two taxa seems to lie in the shape rather that in "classical" distance measurements.

Therefore, the main goals of our study were

1. to find out significant differences between these two taxa both in shell outline and shell indices
2. to compare inter- and intraspecific variability of populations of the two taxa
3. to observe the influence of allometric variation on the results of morphological comparison
4. to compare the results of EFA and „classical" landmark-based analysis.

MATERIAL AND METHODS

Both living animals and empty valves were sampled from 16 sites in the Czech Republic (6 sites inhabited by *S. nucleus*, 9 by *S. corneum*; see the list of collection sites in Table 1). The shape of kidney, shell porosity and arrangement of scars of siphonal retractors were used as the main characters for species determination. Altogether, 329 valves were used for the analyses.

Table 1 List of collection sites

Name of collection site	Species	Latitude	Longitude	n
Alba	*S. corneum*	50°09′09′′	16°05′08′′	20
Bechyňský potok	*S. corneum*	49°14′0′′	14°39′20	41
Cidlina	*S. corneum*	50°7′19′′	15°10′3′′	20
Dírenský potok	*S. corneum*	49°14′56′′	14°45′21′′	20
Horka	*S.nucleus*	49;37′30′′	17°14′	9
Kačení louka	*S.nucleus*	49°45'04′′	16°59′47′′	9
Libice	*S.nucleus*	50°6′42′′	15°9′45′′	11
Lipno	*S.nucleus*	48°41′	14°06′	15
Maškův mlýn	*S. corneum*	49°59′55′′	14°19′05′′	26
Poodří	*S.nucleus*	49°42′23′′	18° 05′15′′	14
Popovice	*S.nucleus*	50°15′25′′	15°40′46′′	20
Rokytka	*S. corneum*	50°04′43′′	14°36′48′′	35
Sánský kanál	*S. corneum*	50°11′24′′	15°05′38′′	21
Tvrdonice	*S.nucleus*	48°44′58′′	17° 00′14′′	31
Vidnava	*S. corneum*	50°22′59′′	17°12′00′′	20
Vltava	*S. corneum*	50°05′37′′	14°25′02′′′	27

Preliminary tests showed the images of left valves to be undistinguishable from the inverted images of the right ones for each pair of valves coming from the same animal and thus only one of each pair can be used in the analysis without loose of any important information. For further work, only left valves (which were found more numerous in the samples of separated empty valves) were taken into account. Frontal and lateral images of the shells were taken with scanner (1200dpi), always with the same magnification and with a ruler aside each shell. The program TPSDig (Rohlf 1999) was used to draw and process outline curves. The outline curves created in this program consisted each of 50-60 points spaced as equidistantly as possible along the image outline (except for the area near umbones, where they had somewhat higher density).

The transformation of image outlines into sum of harmonic functions was done in Size, position and rotation was assigned as invariants. The first six harmonics were used for statistical analyses, whereas the first three parameters of the first harmonic were close to 0 or 1 due to standardization an therefore omitted.

For classical morphometrics, the three dimensions – length (L), height (H) and width (W) were measured to the nearest 0.1 mm using slide calliper. Two additional measurements - hinge length (HL - distance between the prominent parts of cardinal teeth) and hinge height (HH) were measured under stereomicroscope with eyepiece micrometer and the indexes H/L (heigth index), W/L (obesity), W/H (roundness index), HH/L and HL/L were calculated. VBA Data Analysis Tool in MS Excel was used to calculate the constants of logarithmic equations of growth for the respective populations.

Both types of data were then analysed using software packages Statistica 6.0 and Canoco 4.5 for Windows. PCA and DA (grouping variable "species") were performed to find out whether any clusters formed by species or populations can be observed and to compare the variability explained by the particular variables. To visualize the assumed allometry, specimens from selected populations (underlined in the Table 1) were divided into length classes with range of 2 mm and the ordination of the length classes on the PCA biplots was observed. To eliminate the influence of allometric growth, PCA with shell length as covariate was performed.

Variability decomposition was performed using redundance analysis (RDA) in Canoco for Windows 4.5.

RESULTS

Shell indices and their relations

Sphaerium corneum and *Sphaerium nucleus* show significant differences in mean values of shell length, height and width (t-test: $p<0.001$). For ranges and averages of the respective measurements, see Table 2

In the case of the roundness index and "obesity", there is a great variance of the individual values, which are significantly higher in the populations of *S. nucleus*. The wide range of individual values of these indices is probably caused by allometric relations between the variables L, H, and W, which can be expressed in form of the logarithmic equations (see Table 3).

Results of PCA, DA and RDA

As frontal images did not yield any considerable differences between the two taxa, only the lateral ones were used for further analyses.

Both the landmark-based data (indices H/L, W/L, HL/L and HH/L) and the outline-based data yielded two widely overlapping clusters in PCA (see Figs 1 and 2). Neither of the populations was well separated and defined. When using shell indices, the first two PCA axes explain 68.5% of the total variability and are most correlated with the indices W/H, W/L and HL/L, whereas the cluster of *S. nucleus* is oriented towards higher values of these indices. The two taxa could not be separated using DA (Wilks' Lambda: 0.6569550, approx. $F(5,313) = 32.68811$; $p < 0.0000$). When applying PCA and DA to outline-based data, the respective values are: 48.3%; Wilks' Lambda:= 0.401; approx. $F(21,184) = 13,10$; $p < 0.0000$.

After the categorization by size, the representatives of the respective length classes form clusters in the PCA biplot; these are more distinct in the case of outline-based data.

Performing PCA with shell length as covariate resulted in somewhat better distinction between clusters of the two taxa, however, some overlap still occurred, particularly in the case of landmark/based data.

The results of RDA are illustrated by Figures 5 and 6, showing the considerable (10, respectively 24 %) percentage of variability, which can be explained by shell length, i.e. considered as an effect of allometric growth.

DISCUSSION

Both landmark-based and outline-based morphometrics revealed certain differences between the two supposed species. Disregarding of whether they really have the status of distinct species or just ecological forms, they are morphologically distinguishable. The results of distance measurements are in correspondence with the findings of the previous authors, only the range of W/L and W/H is much wider than reported by Korniushin (1994) (see Table 1), who apparently did not use young specimens for his investigations (as the low values of the indices are typical for very small individuals). *S. nucleus* can really be described as “more rounded” (having higher values of W/L, W/H) with broad hinge plate (higher values HL/L) and also growing more quickly in the dimension “W” in comparison with *S. corneum*. However, the variance of the individual values is so large that it makes it impossible to use any of the indices as a reliable character for determination - this would be meaningful only where some dimensions, or their proportion, remain constant in the entire lifespan of the animal. Taking into account the differences between *S. corneum* and

S. nucleus in the shell width, it is surprising that the frontal images, which should reflect the influence of W/L and W/H, did not reveal any remarkable difference between the two taxa.

If the success of landmark-based and outline-based morphometrics in combination with EFA-PCA/DA should be compared, the image analysis proved better than distance measurements. In both cases, the allometry can be considered as the mean difficulty, but the EFA method is less sensitive to it.

To conclude, neither landmark-based nor outline-based morphometrics can be used for species determination but the latter can be used to reveal differences often undistinguishable by naked eye.

Table 2 - values of shell indices

Index (range; average)	Species	Holopainen and Kuiper 1982	Korniushin 1994	Present study
H/L	*S. corneum*	0.78 - 0.82	0.8-0.85	0.68-0.89
	S. nucleus		0.82-0.86	0.77-0.89
W/L	*S. corneum*			0.38-0.73
	S. nucleus			0.48-0.83
W/H	*S. corneum*	0.52-0.86 (W/2H ε(0.26;0.43))	0.67-0.8	0.47-0.91
	S. nucleus		0.72-0.88	0.58-0.98

Table 3 Coefficients of the equations of allometric growth logH′=alogL+logb logW=clogL+logd

Population	a	b	c	d
Alba	0.94	0.94	1.19	0.51
Bechyňský potok	0.97	0.87	1.39	0.21
Cidlina	0.91	0.98	1.30	0.41
Dírenský	1.01	0.82	1.35	0.29
Lipno	0.88	1.04	0.98	0.56
Maškův mlýn	0.96	0.85	1.42	0.22
Rokytka	0.96	0.87	1.26	0.35
Sánský kanál	0.88	1.04	1.25	0.33
Vltava	0.94	0.91	1.08	0.45
S. corneum - Alimov 1967	1.18	0.58	1.18	0.47
S.corneum - Korniushin 1994			1.3	0.42
Horka	0.95	0.94	1.184	0.51
Kačení louka	0.94	0.98	2.18	0.5
Popovice	1.06	0.75	1.72	0.18
Poodří	0.96	0.90	1.56	0.22
Tvrdonice	1.00	0.82	1.47	0.25
S. nucleus - Korniushin 1994			1.53	0.3

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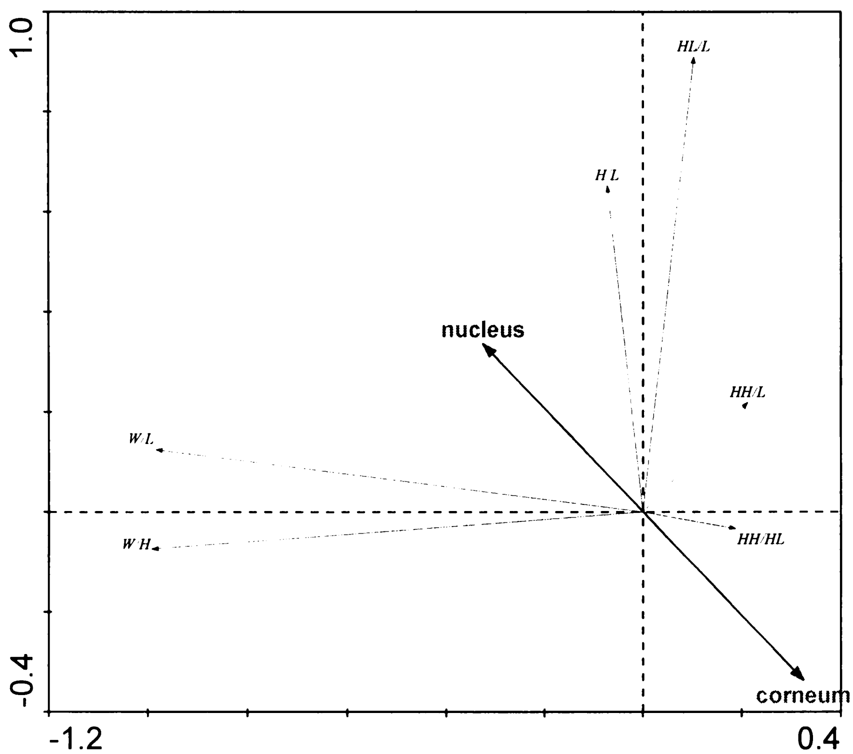
Figures



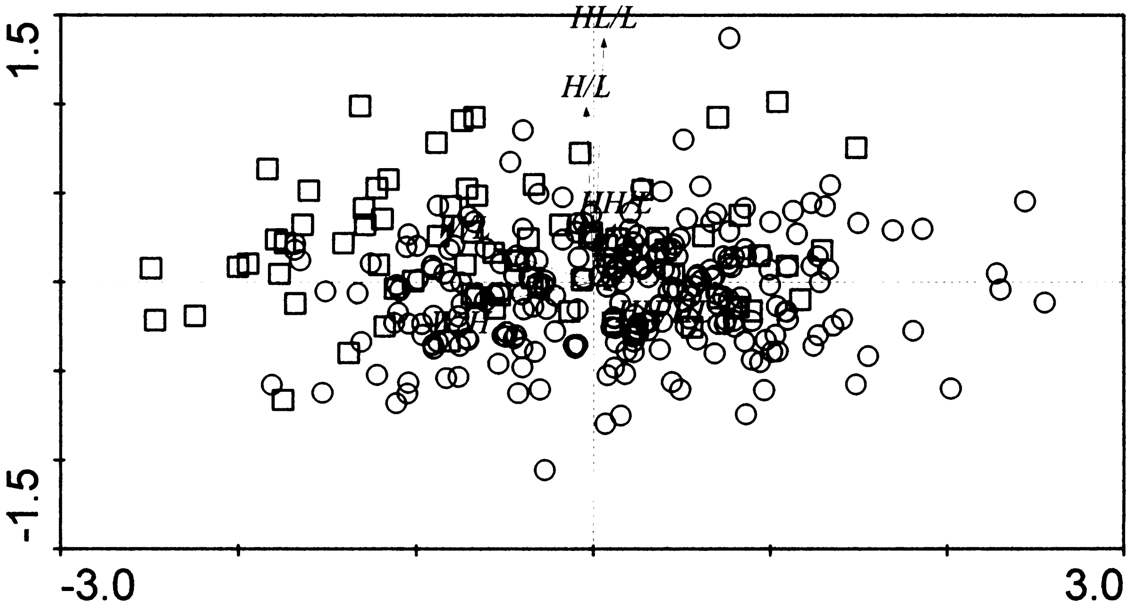


Fig. 1.: PCA ordination plot of shell indices (squares = *S. nucleus,* circles = *S. corneum*)

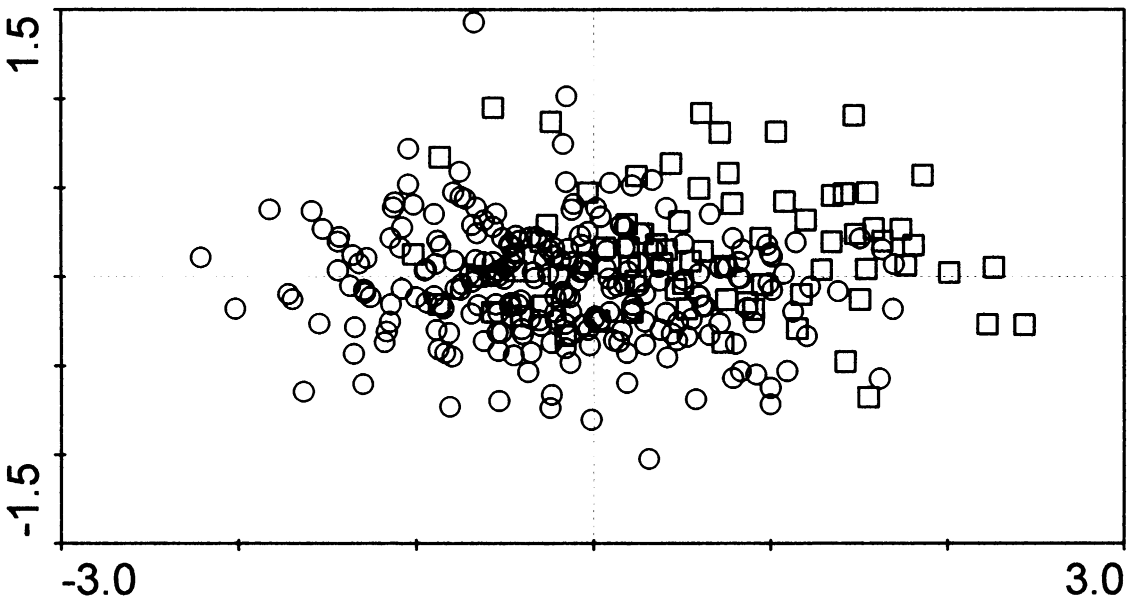


Fig. 2: PCA scatterplot of EFA factors (squares=*S. corneum*, circles = *S. nucleus*)

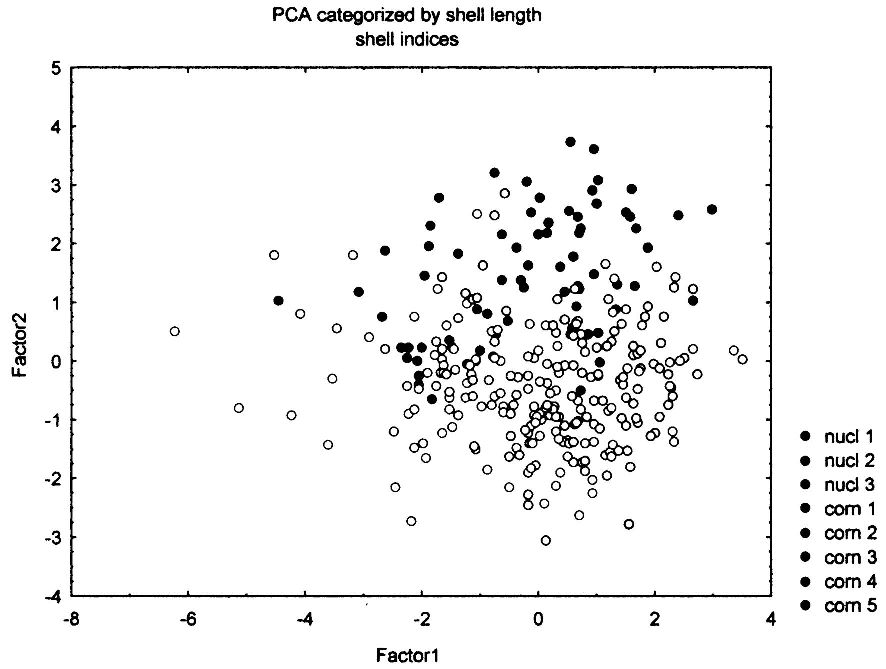


Fig 3: PCA categorized by shell length (full circles = *S. nucleus*, empty circles = *S. corneum*)

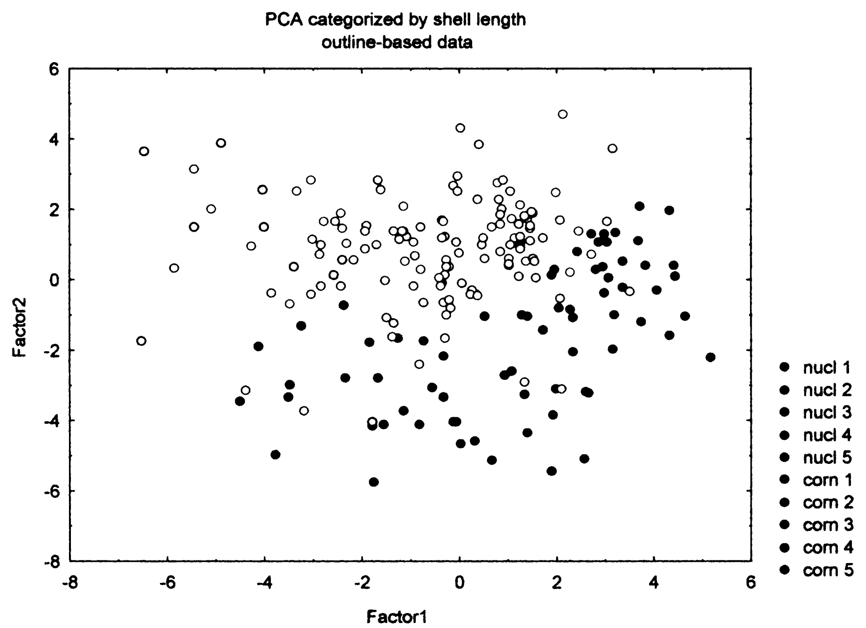


Fig. 4: PCA categorized by shell length (full circles = *S. corneum*, empty circles =*S. nucleus*)

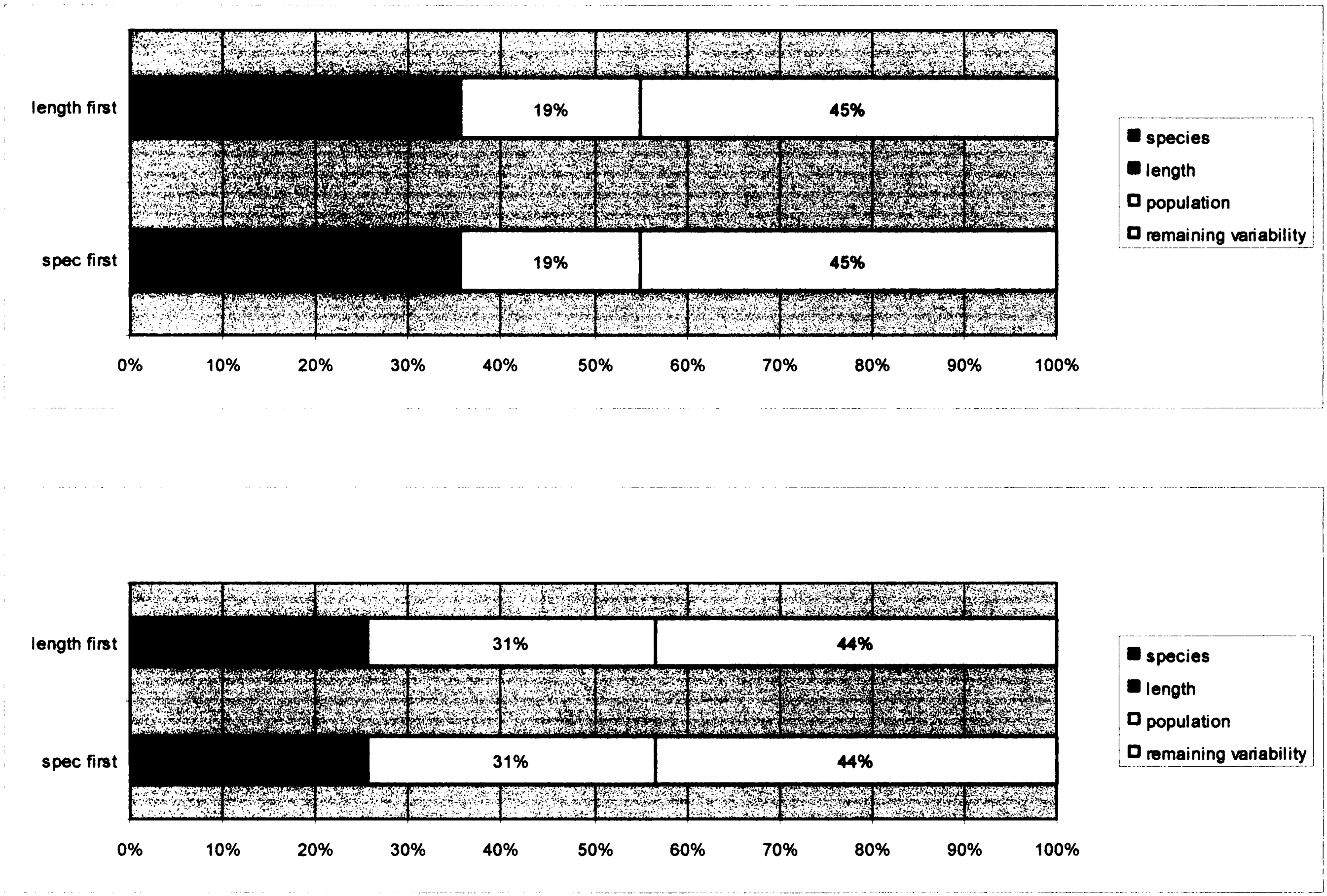


Fig. 3: RDA analysis based on outline-based data (above) and landmark-based data (below)

CHAPTER 4

Karyology

(after some additions the article will be offered to Journal of Molluscan Studies)

COMPARISON OF KARYOTYPES OF SIBLINGS *SPHAERIUM CORNEUM* AND *SPHAERIUM NUCLEUS*

INTRODUCTION

The family Sphaeriidae is peculiar for frequent occurrence of polyploidy, presumably allopolyploidy as could be assumed from the formation of normal bivalents in the meiosis of polyploid species (Lee 1999). Only European species *S. nitidum* (2n=30 - Barsiene & Baršytė 1996a) and *S. corneum* (2n=30 - Baršienė &Baršytė 1996, 2n=36 - Keyl 1956) were considered as diploid so far. Thus, the question arises which of the chromosome numbers ascribed to *S. corneum* is correct. I am most likely to believe that the higher chromosome number as reported by Keyl was either due to some genotoxic effect of environment (Baršienė & Baršytė 2000) or due to the confusion of *S. corneum* with some other species.

In past, the taxon *S. corneum* comprised both *S. corneum* s. str. and *S. nucleus*, which is now regarded as a distinct species. However, no reference of karyotype of the latter species has been published yet. The detailed karyological investigation of both taxa could therefore elucidate the whole problem of their distinctness.

MATERIAL AND METHODS

Living animals belonging to *Sphaerium corneum* and *Sphaerium nucleus* (distinction between the two was made according to the internal anatomy) were sampled from three collection sites (mill-race near Maškův mlýn, Bechyňský potok - *S. corneum*; small pools near Studénka in Poodří Protected Landscape area - *S. nucleus* - for detailed description see Appendices) using bowl-shaped sieve and transported to the laboratory. About 20 specimens of each species were used. At least 48 hrs. after collection, the pieces of gonads or embryos were dissected out and chromosome preparations were made. As immersing the living animals into 0.01% and 0.02% solution of colchicine did not result in higher mitotic index in chromosome preparations, this step was excluded from the most experiments. Pieces of tissue were hypotonized in distilled water for 20-40 minutes, fixed in methanol-acetic acid (3:1) for 15 min. and dissociated in 60% acetic acid. Cells were smeared on slides heated up to 40°C on the hot plate. The slides were stained with 5% Giemsa prepared on Sorensen buffer (pH 6.8) for 40 minutes.

RESULTS

About 25 mitotic metaphases of each species were observed (including the incomplete ones).

Although hypoploid nuclei (2n=26-29) or figures with 1 or 2 additional small chromosomes could be observed, the most frequently observed chromosome number in both species was 2n=30. *S. nucleus* possess 12 pairs of metacentric and 3 pairs of submatecentic chromosomes, whareas *S. corneum* has only metacentric chromosomes. The last pair of chromosomes in *S. nucleus* is strikingly small (relative chromosome length less than 0,15%)

Table 1 - chromosome measurements of *S. corneum* and *S. nucleus* (2n=30)

Sphaerium corneum				*Sphaerium nucleus*			
Chromosome pair no.	relative length (%)	length of short arm/length of long arm	chromosome type	Chromosome pair no.	relative length (%)	length of short arm/length of long arm	chromosome type
1	5.8	1.22	metacentric	1	7.01	1.23	metacentric
2	5.3	1.01	metacentric	2	6.2	1.27	metacentric
3	5.03	1.33	metacentric	3	4.6	1.1	metacentric
4	4.8	1.69	metacentric	4	3.7	1.33	metacentric
5	4.5	1.24	metacentric	5	3.7	1.21	metacentric
6	4.12	1.12	metacentric	6	3.6	1.4	metacentric
7	3.8	1.1	metacentric	7	3.6	1.61	metacentric
8	3.2	1.54	metacentric	8	3.4	1.72	submetacentric
9	2.7	1.08	metacentric	9	3.3	2.02	submetacentric
10	2.5	1.23	metacentric	10	3.3	1.45	metacentric
11	2.5	1.35	metacentric	11	3.3	1.69	metacentric
12	2.5	1.02	metacentric	12	3.1	1.96	submetacentric
13	2.5	1.07	metacentric	13	2.8	1.504	metacentric
14	2.2	1.38	metacentric	14	0.7	1.607	metacentric
15	1.88	1.25	metacentric	15	0.3	1.05	metacentric

DISCUSSION

The most frequent chromosome number 2n=30 in both species is the same as in *S. nitidum* (Baršienė and Baršytė 1996) and quite in correspondence with the presuposition made by Baršienė et al. (1996b), that the common chromosome numbers in the family Sphaeriidae are multiples of 30. The frequent occurrence of hypoploid cells or additional chromosomes might be to some measure considered as an artefact of the preparation, but presence of abnormal karyotypes is quite common in molluscs (Baršienė et al. 1998, Baršienė &.Baršytė 1996a) and might be increased by genotoxical effects of the environment.

The differences in morphology (12 pairs of metacentric and 3 pairs of submetacentric chromosomes in cells of *S. nucleus* vs. 15 metacentric pairs in *S. corneum*) and relative lengths allows to speculate about existence of reproductive barriers between the two studied taxa. However, comparative study of more populations would be necessary to compare stability of the above mentioned karyological characters.

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FIGURES

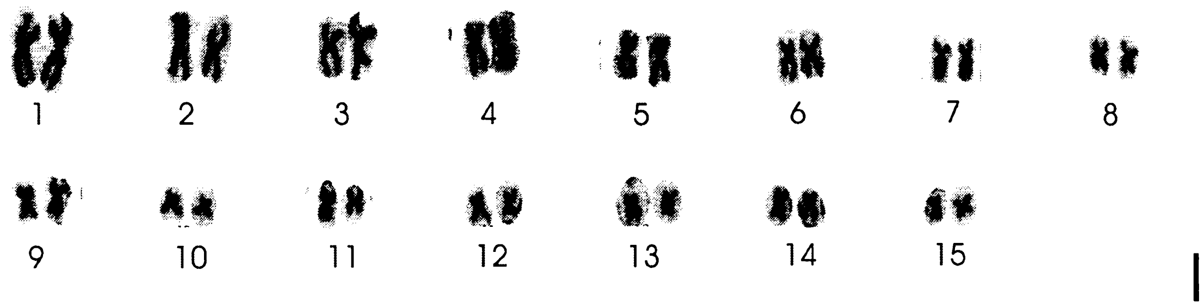


Fig. 1 : Modal karyotype of *Sphaerium corneum* (scale bar=10μm)

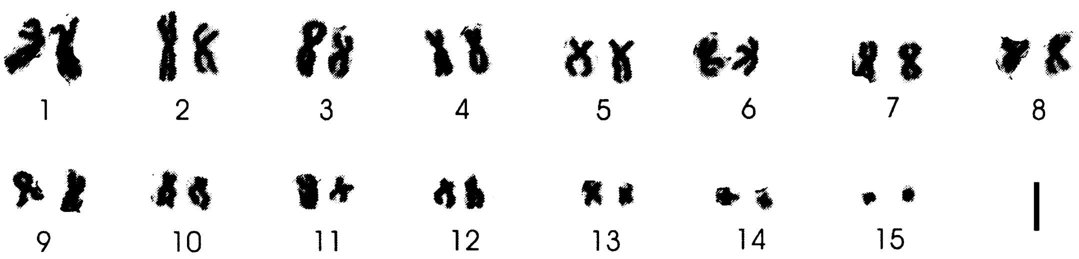


Fig. 2: Modal karyotype of *Sphaerium nucleus* (scale bar=10μm)

CHAPTER 5

Sphaerium nucleus vs. *Sphaerium corneum* – sibling species or not? (Published abstract)

Kořínková, T. (2006): Sphaerium corneum and Sphaerium nucleus - sibling species or not? The Malacologist 46: 13-14

Sphaerium nucleus vs. *Sphaerium corneum* – sibling species or not?

Sphaerium nucleus (Studer 1820) has long been regarded as a subspecies or form of *S. corneum*. It was first Korniushin who gave convincing proofs (based on not only conchological, but also anatomical and ecological characters) of its distinctness.

In the last years, a few localities where of *S. nucleus* occurs have been found in the Czech Republic. Material of both *Sphaerium corneum* and *Sphaerium nucleus* was examined anatomically and histologically, the study being focused mainly on the shell pore density, shell shape, nephridium and other details of internal anatomy (e.g. the so-called "caecal cells" of the mantle epithelium, which were found to be more numerous in *S. nucleus*). In my material, the main characters originally proposed by Korniushin as stabile throughout the Palaearctic region, were sufficient to distinguish individuals of S. nucleus from those of S. corneum. However, in some populations more or less frequent exceptions and irregularities (namely in the arrangement of nephridium and scars of siphonal retractors) were also observable. Which of the characters are good enough to give some taxon the range of a species is a point for discussion

GENERAL CONCLUSION

Populations of *Sphaerium corneum* and *Sphaerium nucleus* from the Czech Republic were studied by means of morphometrics, anatomy, histology, karyology and additional environmental variables were measured in order to answer the question, whether the two taxa are sibling species. The problem of sibling species is, in other words, the problem of different species concepts. The term is generally used for morphologically nearly identical species, which are reproductively isolated from each other, i.e. which are distinct according to the biological species concept but not according to typological species concept.
From the present analyses and observations, it can be concluded, that:

- The morphometric shell characters are really unreliable for discrimination between the two taxa. Although some differences both in outlines and shell indices occur, there is indispensable overlap.
- Shells of S. nucleus have significantly higher pore density than those of S. corneum. This character is relatively stable among all the populations belonging to the species. However, the strong correlation between the pore density and the amount of dissolved oxygen in the critical period of year leads to doubts whether this character is determined genetically or just by environment.
- There are other characters probably related with environment – the density and length of caecal cells of the outer layer of mantle epithelium, the length of siphons and strength of siphonal retractors, the number of embryos per brood pouch. In all of them, transitional forms are not rare.
- The shape of kidney is the most obvious character, but with certain phenotypical plasticity.
- If any outstanding difference in karyotypes was found, the two taxa could be really regarded as incapable of interbreeding and their distinctiveness would be supported. However, the karyotypes of the two taxa show identical chromosome number and rather similar morphology.

The implications for status of *S. corneum* and *S. nucleus*:

- Further molecular and cytogenetical experiments are needed to support their specific distictiveness.

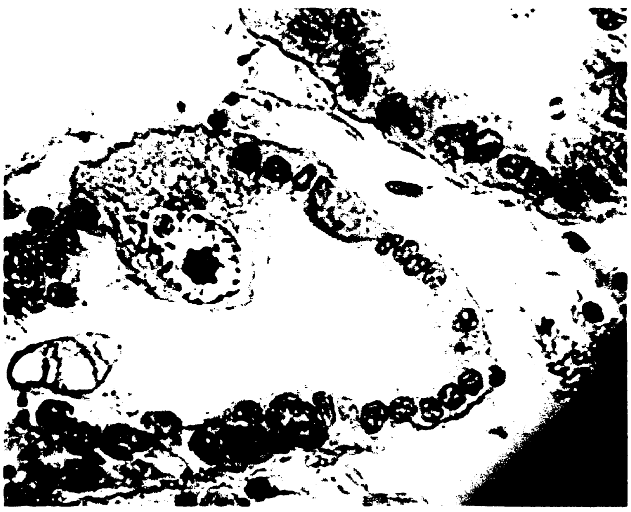
- Unless this is done, the both can be regarded just as ecological species or morphospecies.

Appendix

Histological sections

1

2



3

***Sphaerium corneum* – histological sections (paraffin, Masson´s triple stain):**

1 – frontal section (scale bar = 1 mm)

ft – foot; id – inner demibranch, int – intestine, la – larva, od – outer demibranch

2 – larva in the brood pouch (bp) of the adult; mt – mantle of the larva, ft – foot of the larva;

st – stomach

3 – section through ovarium with developing oocyte (oc) (scale bar =0.1 mm)

Summarized list of all collection sites

Alba 50°09′09′′ ;16°05′08′′ CZ-5862a-b- the Alba race N of Týniště nad Orlicí, running water, bottom: sand, mud, leaves and other organic material, width ca. 2 m, depth at collection site ca. 40 cm.
Sphaerium corneum, high abundance (10-20/m^2) (25 specimens dissected, 31 shells measured, 20 shells photographed) col. T. Kořínková
Other molluscan species: *Pisidium amnicum, Radix auricularia*

Bechyňský potok 49°14′0‘‘; 14°39′20′′ CZ-6753d- Bechyňský potok near the road bridge, ca. 2 km S of Záluží, running water, coarse-grained (particles ca. 2 mm in diameter) sand and partly silt. width ca. 150 cm, depth 20 cm
Sphaerium corneum high abundance (over 20/m^2) (10 specimens for molecular techniques, 25 specimens dissected, 41 shells photographed)

Other species: *Radix auricularia*

Cidlina 50°7′19′′; 15°10′3′′ CZ-5857c – river Cidlina ca 2 km SW of Libice nad Cidlinou, near the bridge, bottom: presumably sand, width ca. 10m, depth at collection site 0, 7 m
Sphaerium corneum (low abundance, <5/ m^2) (20 specimens dissected, 39 shells measured, 20 shells photographed)

Other molluscan species: *Bythinia tentaculata, Radix peregra, Galba truncatula, Physella acuta, Unio pictorum, Unio tumidus, Sphaerium rivicola*

Dírenský potok 49°14′56′; 14°45′21′′ CZ-6754d - Dírenský potok near the bridge in the village Přehořov, running water, bottom: stones, sand, width ca 5 m, depth 30 cm
Sphaerium corneum (low abundance, <5/ m^2, restricted to sand between blocks) (20 specimen dissected, 20 shells measured, 10 specimen for molecular biology)

Other molluscan species: *Unio tumidus, Pisidium casertanum, Pisidium subtruncatum*

Kačení louka 49°45’04’’′; 16°59’47” CZ-6267, small pools (length and width of the largest one ca. 15 and 3m respectively, max. depth 0.7 m) with dense vegetation cover, Litovelské Pomoraví Protected Landscape Area, near the railway ca. 1.5 km E of Moravičany, bottom: silt, organic material
Sphaerium nucleus (<1/ m^2) (2 specimen dissected, 9 shells measured and photographed)

Kotvice 49°42’23”; 18° 05’15” CZ-6274, Small drain (width 0.5 m, depth 0.5 m) with slowly running water and muddy bottom, partly covered with water plants; near the pond Kačák, Poodří Protected Landscape Area, ca. 1.5 km SE of Studénka,
Sphaerium nucleus (high abundance (>100/ m^2), but burrowed deep into sediment in some periods of year) (14 shells measured and photographed, 10 specimen for molecular techniques, 15 specimens dissected)

Libický luh 50°6′42′′ 15°9′45′′ litoral of a pool in a flood-plain forest near Libice nad Cidlinou, depth at collection site 10-20 cm
S. nucleus (15 specimen dissected, 12 shells photographed), collected by L.Beran

Other molluscan species: *Acroloxus lacustris, Stagnicola corvus, Planorbis planorbis, Bathyomphalus contortus, Segmentina nitida, Planorbarius corneus, Lymnaea stagnalis*

Maškův mlýn 49°59′55′′; 14°19′05′′ CZ-6051a-b , old mill-race connected to Radotínský brook, ca. 2 km SW of Praha-Radotín, Český kras Protected Landscape Area, stagnant or

running water depending on the season, bottom: silt, leaves and organic material, width 1 m, max. depth 0, 5 m
Sphaerium corneum (high abundance > 100/ m^2) (10 specimen for molecular techniques, 26 photographed)

Popovice 50°15′25′′ 15°40′46′′ CZ-5760a, 2 small (ca. 3 m in diameter) pools near Bystřice river, on the verge of village Popovice, stagnant water (site dry on the date of collection) with dense vegetation cover, reed, bottom: silt and organic material
Sphaerium nucleus (>10/ m^2) (20 shells measured and photographed, 10 specimen dissected, 10 specimen for molecular techniques)

Rokytka 50°04′43′′ 14°36′48′′ CZ-5953a-b, brook Rokytka close to the built-up area of Praha-Běchovice, running water, bottom: silt, gravel and stones, width ca. 2 m, max. depth 0, 7 m
Sphaerium corneum (irregular distribution, somewhere > 50/ m^2) (10 specimens for molecular techniques, 27 specimens dissected, 35 shells photographed)

Sánský kanál 50°11′24′′ 15°05′38′′ CZ-5856a-b- Sánský kanál between Křečkov and Budiměř, running water, bottom: sand, mud, ca. 10 cm layer of leaves, small picks and other org. Material, width ca 150 cm, depth at collection site 30 cm.
Sphaerium corneum (irregular distribution, somewhere > 30/ m^2) (20 specimens dissected, 21 shells photographed)

Tvrdonice48°44'58''; 17° 00'14'', , CZ-7268 Water ditch (width at the collection site ca. 1 m, depth 0.5 m) with nearly stagnating water, muddy bottom and edges covered with water plants; approx. 2 km SE of Tvrdonice, S Moravia,
S. nucleus (irregular distribution, somewhere > 20/ m^2) (10 specimen for molecular techniques, 15 specimens dissected, 31 shells photographed)

Other molluscan species: *Planorbarius corneus, Planorbis planorbis, Anisus vortex*

Vltava 50°05′37′′ 14°25′02′′ CZ-5952a-b, littoral zone of the Vltava river in Prague, near Čechův most (road bridge), bottom: sand, depth at collection site ca. 1 m
Sphaerium corneum (12+5 specimens dissected, 25 shells photographed)

low abundance (<5/ m^2)

Vidnava 50°22′59′′ 17°12′00′′ CZ-5669a water running from the wetland ca. 0,5 km N of Vidnava, max. width 1 m, depth 0, 5 m
S. corneum (high abundance > 50/ m^2) (10 specimen for molecular techniques, 20 shells measured and photographed)

Žabakor 50°33′0′′ 15°3′3′′ CZ-5456d - 5 specimens dissected, collected by L.Beran

The present distribution of *Sphaerium nucleus* in the Czech Republic