Eurasia Specialized Veterinary Publication

Entomology Letters

2021, Volume 1, Page No: 5-10

Available online at: www.esvpub.com/

A Comprehensive Analysis of the Silphidae Family (Coleoptera: Silphidae) in the Greater Caucasus Ecosystem

Sergey Pushkin[1]*, Bohdan Tsymbal[2], Sergey Artemev[2], Galyna Fesenko[3], Tetiana Fesenko[4]

[1]Department of General Biology and Biodiversity, Institute of Living Systems North Caucasus Federal University, Stavropol, Russia.
[2]Department of Occupational, Technogenic and Environmental Safety, National University of Civil Defence of Ukraine, Ukraine.
[3]Department of History and Cultural Studies O. M. Beketov National University of Urban Economy in Kharkiv, Ukraine.
[4]Department of Automation and Computer-Integrated Technologies, Kharkiv Petro Vasylenko National Technical University of Agriculture, Kharkiv, Ukraine.

***E-mail** ✉ sergey-pushkin-st@yandex.ru

ABSTRACT

Unlike larger families such as Carabidae, Chrysomelidae, Scarabaeidae, and Staphylinidae, the Silphidae fauna has not been extensively studied. Given the unique characteristics of the Caucasian fauna, conducting a comprehensive inventory of Silphidae species and subspecies in the Caucasus remains a crucial objective. This study summarizes 23 years of investigations into the carrion beetle fauna (Coleoptera: Silphidae) of the Greater Caucasus. The primary collection method involved manual gathering from carcasses (for necrobionts), which proved effective. Bait traps also yielded positive results. Alongside these techniques, we employed basic entomological equipment, including an entomological sieve, spatula, tweezers, and knife. The species composition of Silphidae beetles is influenced by temperature parameters, with both extreme heat and cold limiting the larval development rate. Silphids typically undergo a one-year life cycle, overwintering in the pupal stage. Humidity plays a significant role in determining the distribution of species strongly linked to carrion. The work provides an eco-faunistic overview, documenting twenty-seven species and four subspecies within the Silphidae family.

Keywords: Carrion beetles, Silphidae, Greater Caucasus, Ecosystem

Received: 10 April 2021
Revised: 04 August 2021
Accepted: 11 August 2021

How to Cite This Article: Pushkin S, Tsymbal B, Artemev S, Fesenko G, Fesenko T. A Comprehensive Analysis of the Silphidae Family (Coleoptera: Silphidae) in the Greater Caucasus Ecosystem. Entomol Lett. 2021;1:5-10.

Introduction

Unlike larger families such as Carabidae, Chrysomelidae, Scarabaeidae, and Staphylinidae, the Silphidae fauna has not been extensively studied. Given the unique characteristics of the Caucasian fauna, conducting a comprehensive inventory of Silphidae species and subspecies in the Caucasus remains a crucial objective. This requires a detailed taxonomic framework, access to comparative specimens from neighboring and distant regions, and, in some instances, an examination of nomenclatural types. Our research incorporates collections and studies from the Caucasus region, along with reviews of the family [1, 2] and other relevant works [3-15] addressing the fauna of the Caucasus and surrounding areas, enabling us to organize the existing information. Additionally, our earlier studies [3-9, 16-18] have focused on silphid fauna and necrobiotic insects within Caucasian territories.

Although specialized studies were lacking, data on the region's fauna were gathered from the early 19th century through the 1990s. Pallas [19] was the first to report on carrion beetles in southern Russia, identifying 6 species. Reliable documentation on the Silphidae fauna in Ciscaucasia and northwestern Caucasus can be found in studies [20-22]. Previous investigations have also explored the Coleoptera fauna of the Caucasus [10, 23-26]. The catalog [11] lists twenty-four species and five subspecies recorded within the current boundaries of the study area.

Zaitsev [12] provided a summary of the fauna from the Caucasus and neighboring regions, but his work contained several inaccuracies, some of which were highlighted in Shavaller's revision [27]. For instance, Zaitsev treated the endemic Caucasian species *Thanatophilus armeniacus* Reitter, 1912 as equivalent to T. porrectus Semenov 1891 [27]. A similar issue arises in his identification of Armenian species, drawing parallels with the account of a lamellar beetle from the genus *Trochaloschema* Reitter, endemic to Hissar-Darvaz—*T. armeniaca* Brenske, 1897 [2]. Both species likely originated from the same mislabeled specimens from a common source. Additionally, several names were misinterpreted [28]. For example, *Silpha obscura nitida* Portevin, 1907, imported from the Himalayas, is unsuitable for Caucasian subspecies. Certain names proposed by Menetries (*S. obscura* var. *costata** Men., 1832 and *S. obscura* var. *striola* Men., 1832) are associated with forms from the Caucasus and Transcaucasia and cannot be linked to the nominative subspecies. Furthermore, *Silpha costata* Men., 1885, identified by Reitter [10] as an intraspecific form of *Silpha tristis*, supports the Caucasian subspecies designation of *S. obscura striola* Menetries 1832 (synonyms: *S. obscura nitida* Portevin 1907; Schawaller, 1980) [2].

A brief review of the propagation of Nicrophorini in the Palaearctic region is provided in Semenov-Tyan-Shansky [29]. Studies in papers [30, 31] focused on Ciscaucasia, but they did not offer an analysis of species distribution, ecological characteristics, or synonymy, which led to some inaccuracies. Species such as *Aclypea sericea* Zubk. and *A. tomentifera* Rtt. are usual in the mountainous areas of the Caucasus and Transcaucasia, while *Necrodes littoralis* L. and *Nicrophorus humator* Gleditsch are found in the semiarid regions of Kalmykia. The latest revision [32] places four species within the genus *Ablattaria*. However, according to Nikolaev and Kozminykh [2], many of these should not be considered separate species. In the Caucasus, a polytypic species is widespread, and a clear boundary between subspecies areas is difficult to establish. Occasionally, individuals with noticeably larger dots on the elytra are found in Crimea, but such specimens are more frequent as one moves eastward. In the Grozny region, only typical instances of *A. laevigata cribrata* (Mén., 1832) have been observed, with the major dot on the elytra several times larger than the others.

The adults and larvae of carrion beetles play a vital role in the trophic dynamics of terrestrial biomes in temperate zones. In arid regions, members of the genera *Silpha*, *Thanatophilus*, and *Nicrophorus* face competition from skin-eating and other xerophilous scavengers, such as those from the families Trogidae and Nitidulidae, for food resources. Silphidae acts as primary consumers, necrophages, and predators, engaging in phoresis with nematodes and gamasid mites. They help control populations of sluggish insects and mollusks (such as *Dendroxena*, *Ablattaria*, and *Phosphuga*), which are pests in fields and forests and contribute to soil formation, especially *Nicrophorus* [3-9].

Materials and Methods

This study primarily relies on our data collected across various regions of the Caucasus over 23 field seasons (1994-2017) during the spring and autumn months. The alpine areas were examined through several walking expeditions that traversed the primary mountain ranges of the Caucasus, from the foothills to the subnival zone.

The primary collection method involved manual gathering from carcasses (for necrobionts), which proved effective. Bait traps also yielded positive results. Alongside these techniques, we employed basic entomological equipment, including an entomological sieve, spatula, tweezers, and knife. All species found on loose (soil, wood dust, moss) or semi-liquid (excrement, decaying mushrooms, partially decomposed bodies) substrates were collected on a polyethylene sheet (1m²). “Barber” traps (soil traps) were used as well; these consisted of plastic cups (0.5 L) filled with a preservative liquid (80% ethanol solution).

Results and Discussion

The study of carrion beetles in the Greater Caucasus has revealed twenty-seven species and four subspecies. These include *Oiceoptoma thoracicum* (Linnaeus, 1758), *Ablattaria laevigata cribrata* (Ménétriés, 1832), *Necrodes* *littoralis* (Linnaeus, 1758), *Thanatophilus rugosus* (Linnaeus, 1758), *T. sinuntus* (Fabricius, 1775), *T. terminatus* (Hummel, 1825), *T. dispar* (Herbst, 1793), *Silpha obscura striola* (Ménétriés, 1832), *S. tristis* (Illiger, 1798), *S. carinata* (Herbst, 1783), *Dendroxena quadrimaculata* (Scopoli, 1772) = *Silpha quadripunctata* (Schreber, 1759) (non Linnaeus, 1758) [Madge, 1980], *Phosphuga atrata* (Linnaeus, 1758), *Aclypea opaca* (Linnaeus, 1758), *A. undata verrucosa* (Ménétriés, 1832), *A. sericea* (Zoubkoff, 1833), *Nicrophorus germanicus fascifer* (Reitter, 1884) = *N. armeniacus* (Portevin, 1923) [Madge, 1980], *N. germanicus germanicus* (Linnaeus, 1758), *N. humator* (Gleditsch, 1767), *N. vespillo* (Linnaeus, 1758), *N. vestigator* (Herschel, 1807), *N. antennatus* (Reitter, 1884), *N. nigricornis* (Falderman, 1838), *N. fossor* (Erichson, 1837), *N. vespilloides* (Herbst, 1784), *N. investigator* (Zetterstedt, 1824), *N. investigator funeror* (Reitter, 1884), *N. sepultor* (Charpentier, 1825), and *N. confusus* (Portevin, 1924).

The ecological features of the silphid fauna in the Caucasus are outlined below. Oiceoptoma thoracicum was observed across the region, while *Ablattaria laevigata cribrata* was primarily found in open habitats. *Necrodes littoralis* thrived in moist environments, and *Thanatophilus rugosus* was common in humid areas, particularly in the foothill zone forests, steppes, and urban areas. *T. sinuntus* is a generalist species, whereas *T. terminatus* was found in arid regions. *T. dispar* was restricted to forests, mostly on the northern slopes of the Greater Caucasus. *Silpha obscura* was represented by the subspecies *S. obscura striola* which is adapted to meso-xerophilic conditions. *S. tristis* was observed in meadows, and *S. carinata* was found in steppes. *Dendroxena quadrimaculata*, a forest stenobiont, reached its highest numbers in forests over 300 years old. *Phosphuga atrata* was present in forests, occasionally found at altitudes between 2600-3000 m. *Aclypea opaca* was frequently encountered in agrocenosis throughout the region. *A. undata*, represented by the subspecies *A. undata verrucosa*, was observed from the foothills up to 1400 m. *A. sericea*, a rare species, was found in mountainous Dagestan.

Nicrophorus germanicus was represented by two subspecies in the Caucasus: *N. germanicus fascifer* and *N. germanicus germanicus*, with a preference for steppe habitats. *N. humator* was located in forests, while *N. vespillo*, a generalist, was found in a variety of environments. *N. vestigator* inhabited ecotones at the forest edges, and *N. antennatus* was common in open habitats, especially agrocenosis. *N. nigricornis* was restricted to forested areas, while *N. fossor* thrived in meso-xerophilic conditions. *N. vespilloides* was distributed from the foothills to the mid-mountain forest zone. *N. investigator* was located on the northern slopes of the Greater Caucasus, and *N. investigator funeror* replaced the nominative subspecies in Georgia, northern areas of the Greater Caucasus, and Armenia. *N. sepultor* was a mesophilic species, and *N. confusus*, found in mountain-steppe regions, was moderately xerophilic and preferred agrocenosis such as wheat, hay meadows, oats, and deposits. *N. confusus* was noted by Dzhambazishvili in Akhaltsikhe and Akhalkalaki, Georgia, while he reported the absence of *N. sepultor* in the Caucasus. Contemporary views suggest that *N. sepultor* is restricted to the southern foothills of the Caucasus Range [2]. We did not observe *N. confusus* in North Ossetia, and currently, there are no confirmed records of its presence in Russia. The distribution of *N. nigricornis* is limited to the midland areas of the Caucasus, with its peak activity occurring in July and June. *N. confusus* inhabits low- and mid-mountain open regions (at altitudes ranging from 800 to 1500 m), primarily found in intermontane basins shielded from heavy rainfall. It has been recorded in stony pasture meadows up to three thousand meters (Georgia, Trialeti mountain range), and as elevation increases, it is replaced by the subspecies found in the Caucasus (*N. investigator funeror*). *N. sepultor*, a common species in Central and Southern Russia, has not been observed south of the Caucasus Range. *N. littoralis*, *O. thoracica*, *D. quadripunctata*, and *P. atrata* were all recorded in forested areas. *N. littoralis* specifically inhabited moist environments. *P. atrata* and *D. quadripunctata* were recorded in the forest-steppe zones adjacent to forested regions. This distribution is influenced by the hydrothermal conditions and the availability of food sources. *Dendroxena* is primarily found in areas where leaf-eating pests, such as caterpillars of burlaris and gold-winged grubs, thrive, particularly in mature forests that are susceptible to drying out. Phosphuga, known for its widespread presence, thrives in humid environments typically found under forest canopies or near water sources within the steppe and forest-steppe zones. It can also be found at elevations ranging from 2500 to 3000 meters. *A. Clathrina cribrata* was observed in the steppe regions of the Caucasus, with the Stavropol Upland marking one of its northern limits. The more common *A. laevigata* was encountered in the virgin steppes, and both subspecies were recorded in the border region of Stavropol Territory and Chechnya. As we moved eastward, only the *A. cribrata* subspecies were observed.

During the study of Silpha species, it was noted that the distribution of *S. carinata* and *S. obscura* is influenced by hydrothermal factors, with these species not occurring in the eastern Caspian region. *S. tristis* is found in the meadows of the Caucasus. The genus *Silpha* exhibits considerable variability, resulting in numerous morphs and aberrations of species. For example, *S. obscura* shows significant variation, and it has been suggested to be used as a bioindicator of agrocenosis [33]. According to Tikhonov [34], *S. obscura* is a common species in steppe areas affected by overgrazing. *S. striola* is found across the Caucasus and its surrounding countries. The genus *Aclypea* is also widely distributed in the region, with some species being rare in foothill landscapes and meadows. *A. sericea* is primarily found in the western and northwestern parts of Ciscaucasia, although it is more common in Dagestan and Transcaucasia.

Nutritional specialization plays a significant role in the distribution of many species. The largest group consists of necrophagous species, such as *Nicrophorus* and *Necrodes*, which primarily inhabit the carcasses of large mammals like cattle and wild ungulates. These species feed directly on the carcasses. Nicrophorus typically lays eggs on "buried" small mammal carcasses, making it harder to observe. *Necrodes* prefer to lay eggs on medium-sized corpses, usually birds (up to fifteen kg). Phytophagous species like Aclypea feed on plants and, in dry years, can sometimes damage crops. The zoophage group includes genera such as *Phosphuga*, *Dendroxena*, and *Ablattaria*. Many of these genera also exhibit facultative predation. Notably, competition occurs between obligate necrobionts for food, as some beetles feed on the carcasses along with dipterous larvae, while older larvae may prey on the corpse as well. Often, one species will outcompete another for access to the carcass. Trophic specialization among dead-eaters remains an under-researched area, but our observations suggest that many species alter their diet during the imaginal phase. The larva of *Phosphuga atrata* is recorded as a phytophage, while *Necrodes* and *Nicrophorus* consume the eggs and larvae of Diptera. Overpopulation on a carcass sometimes leads to cannibalism, particularly in *Thanatophilus* and *Silpha*. Fungivory has also been observed in *Necrodes*. Coprophagy was noted in Phosphuga and Oiceoptoma, though these instances are infrequent. We discovered *S. carinata* and *Oiceoptoma* on bird pellets. Some claims regarding phytophagy in Ablattaria are likely inaccurate. *T. sinuatus* and *S. obscura* shift to polyphagy during the summer months. Additionally, *Silpha* species have been observed attacking slugs, snails, and other soft-bodied invertebrates.

Based on the gathered data, we have classified the following landscape-ecological complexes. Eurytopobiontic species include *N. vespillo*, *T. sinuatus*, *A. undata*, and *N. humator*, which are some of the predominant species in the Caucasus biotopes. Stenotopic species form the forest complex, which comprises *Oiceoptoma*, *Dendroxena*, and *S. tristis*. This complex consists of species that inhabit the forests and forest steppes of Russia, restricted to the forest zones of the Caucasus. Steppe species, such as *A. cribrata*, *N. germanicus*, and *N. sepultor,* are found in open landscapes, steering clear of forests and dunes. The forest-steppe complex includes P. atrata. Piedmont species, like *N. investigator* and *N. nigricornis*, are typical inhabitants of the Caucasian foothills. Mountain species are represented by *N. funeror* and *N. confusus*. In anthropogenic landscapes, we recorded 11 species. However, none of these species are synanthropic, and like other regions of Russia, they do not adopt a synanthropic lifestyle.

Conclusion

The species composition of Silphidae beetles is influenced by temperature parameters, with both extreme heat and cold limiting the larval development rate. Silphids typically undergo a one-year life cycle, overwintering in the pupal stage [35, 36]. Humidity plays a significant role in determining the distribution of species strongly linked to carrion. If drying occurs too rapidly, larvae cannot utilize the substrate for nourishment, restricting species from entering dry areas where conditions are arid. A comparison of the dead-eater fauna in the Caucasus with neighboring regions highlights its exceptional diversity. This richness is shaped by factors such as the region's varied topography and its unique geographical position near the southern coast. One key contributor to the development of this rich fauna is the presence of effective refuges that enabled stenotopic, thermophilic, and species with narrow ecological tolerances to endure the climatic shifts during the Pleistocene.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Khachikov EA, Arzanov YG. Materials for the fauna of beetles (Coleoptera) of the North Caucasus and the Lower Don. Bug beetles (Silphidae). Fauna and distribution patterns in the region. Rostov; 1990. 14 p.
2. Nikolaev GV, Kozminykh VO. Dead-eating bugs. Almaty; 2002. 158 p.
3. Pushkin SV, Belous VN, Alikhadzhiev MK, Erzhapova RS, Bagrikova NA. Materials on the fauna of micetobiont and mycetophilic beetles (Coleoptera) Ciscaucasus. Entomol Appl Sci Lett. 2019;6(4):1-6.
4. Pushkin SV, Tsymbal BM, Rybalova OV. Use of population indicators of beetle (Coleoptera, Silphidae, Dermes-tidae) in bioindicacion of the environmental status. Entomol Appl Sci Lett. 2019;6(4):13-7.
5. Pushkin SV, Tsymbal BM, Nagdalian AA, Nuzhnaya KV, Sutaeva AN, Ramazanova SZ, et al. The use of model groups of necrobiont beetles (Coleoptera) for the diagnosis of time and place of death. Entomol Appl Sci Lett. 2019;6(2):46-56.
6. Pushkin SV. Environmental group necrophilous and necrobionts beetles (Insecta; Coleoptera) of the south of the Russia. Entomol Appl Sci Lett. 2015;2(4):1-9.
7. Pushkin SV. New data Concerning Mites Phoresia upon Necrophag Bugs. Vestn Stavropol Gos Univ. 2011;74(3):92-6.
8. Pushkin SV. Beetles- necrophages- bioindicators of technogenic pollution of urban system. Mordovia Univ Bull. 2009;9(1):51-3.
9. Pushkin SV. Review of the genus Thanatophilus Leach, 1815 (Coleoptera: Silphidae) of Southern of Russia. Cau Entomol Bull. 2006;(1):41-6. doi:10.23885/1814-3326-2006-2-1-41-46
10. Reitter E. Bestimmungs-Tabellen der europaeischen Coleopteren. XII. Necrophaga (Platypsyllidae, Leptinidae, Silphidae, Ani- sotomidae und Clambidae). Verlag der Verfassers; 1885. 122 p.
11. Jacobson GG. Beetles of Russia and Western Europe. St. Petersburg. 1910;8:596-624.
12. Zaitsev FA. On the distribution in the Caucasus of species of the subfamily Silphini. Izv Kavk Mus. Tiflis. 1914;8(1-2):151-4.
13. Kryzhanovsky OL, Ter-Minasyan ME. Coleoptera. Fauna of the USSR. Vol. 5. Mountainous regions of the European part of the USSR. Moscow – Leningrad; 1958. p. 384-431.
14. Samedov NG. Fauna and biology of beetles harmful to crops in Azerbaijan. Baku; 1963. 383 p.
15. Dzhambazishvili YS. On the use of bait pits with corpses of animals for collecting beetles. Communication. AN Cargo. SSR. Sakartvelos SSR Metsnierebata Akademiis moamba. 1965;40(1):183-6.
16. Pushkin SV. Dead-eating beetles and skin-eating beetles (Coleoptera: Silphidae, Dermestidae) of the Central Ciscaucasia (fauna, ecology, economic importance). Abstract. Dis. Cand. Biol. Sciences. Astrakhan; 2002. 26 p.
17. Pushkin SV. The fauna of dead-eating bugs (Coleoptera: Silphidae) of the mountain Caucasus. Materials of the conference dedicated to the 100th anniversary of S.M. Yablokova-Khnzoryan. NAS of Armenia, Yerevan; 2004. p. 136-7.
18. Pushkin SV. Necrobiont entomocomplex of the highlands of the North-West Caucasus. Eur Entomol J. 2004;3(3):195-202.
19. Pallas PS. Zoografia Rosso-Asiatica, systems animalibum in extenso Imperio Rossico et adjacentibus mariubus observatiorum recensionem domicilla, mores et descriptiones anatomen atqus icones plurimorum. Petropoli (Lipsiae); 1810.
20. Kolenati F. Insecta Caucasi. Coleoptera, Dermaptera, Lepidoptera, Neuroptera, Mutillidae, Aphaniptera, Anopleura. Melet Entomol. 1846;5:1-170.
21. Mannerheim C. Nachtrag zur Kaefer-Fauna der Aleutischen Inseln und der Insel Sitkha. Bull Soc Imp Natural Moscou. 1846;19(2):501-16.
22. Motschulsky V. Observations sur le Musēe Entomologique de l'Universitē Impēriale de Moscou. Article 1. Bull Soc Imp Natural Moscou. 1845;18 (3-4):332-88.
23. Faldermann F. Additamenta entomologica ad faunam Rossicam in itineribus Juessu Imperatoris Augustissimi annis 1827-1834 a cl. Mēnētriēs et szovitz susceptis collecta. Nouv Mēm Soc Imp Natural Moscou. 1835;4:1-314.
24. Faldermann F. Coleopterorum ab illustrissimo Bungio in China boreali, Mongolia, et montibus Altaicis collectorum, nec non ab ill. Turczaninoffio et Stchukino e provincia Irkutzk missorum illustrationes. Mēm Prēs Acad Imp Sci St. Pētersb. 1835;6(2):337-464.

25. Mēnētries E. Catalog raisonnē des objets de Zoologie recueillis dans un voyage au Caucase et jusqu'aux fronti and res actuelles de la Perse entrepris par ordre de SM l'Empereur. Acad Imp Sci St. Pētersb. 1832:271.
26. Reitter E. Bestimmungs-Tabellen der europ d ischen Coleopteren. XII. Necrophaga (Platypsyllidae, Leptinidae, Silphidae, Ani- sotomidae und Clambidae). Verhandlungen des Naturforschenden Vereines in Br ъ nn. 1884;23:3-122.
27. Schawaller W. Taxonomie und Faunistic der Gattung Thanatophilus (Coleoptera Siphi- dae). Stuttg Beitr Naturk Ser A Nr. 1981;351:1-21.
28. Portevin G. Les Grands Necrophages du Globe: Silphini, Necrodini, Necrophorini. Encyclopedie Entomologique. Paris. 1926;6:1-270.
29. Semenov-Tyan-Shansky AP. Classification of grave beetles Coleoptera, Silphidae tribus Necrophorini and their geographical distribution. Tr. Zool Inst USSR Acad Sci. 1932;1/2:149-61.
30. Loktionov PD. P the ground insects of the Don. Rostov; 1981. 140 p.
31. Minoransky VA. Mer measures. Resources of wildlife. Part 3. Insects. Rostov; 1984. p. 69-70.
32. Schawaller W. Revision der Gattung Ablat- taria Reitter 1884 (Coleoptera: Silphidae). Stuttg Beitr Naturkd Ser A Nr. 1979;321:1-8.
33. Kozminykh VO, Yesyunin SL. Dead-eating beetles of the genus Silpha L. (Coleoptera, Silphidae) of the fauna of the Urals. Fauna and ecology of insects of the Urals. Perm; 1990. p. 94-105.
34. Tikhonov AV. Influence of the process of etching on the steppe fauna of invertebrates (on the example of the Rostovsky nature reserve). Actual problems of ecology and nature conservation of ecosystems in the southern regions of Russia and adjacent territories. Krasnodar, KubSU; 2000. 164 p.
35. Kryzhanovsky OL. Family of Dead- Eaters. Insects and ticks pests of agricultural crops. L.; 1974. p. 15-6.
36. Kozminykh VO. Trends in the diversity of carrion beetles (Coleoptera, Silphidae) in the Urals. Zool Mag. 2000;79(2):171-9.