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DISTRIBUTION OF THE SUBTERRANEAN AMPHIPOD *STYGOBROMUS*  
IN CENTRAL COLORADO STREAMS,  
WITH NOTES ON THE INTERSTITIAL COMMUNITY

Steven P. Canton<sup>1</sup> and James W. Chadwick<sup>1</sup>

**ABSTRACT.**—As part of a larger study on the aquatic life of streams in central Colorado, efforts were made to determine the distribution of interstitial organisms, with emphasis on the subterranean amphipod *Stygobromus*. In a preliminary screening-level study, sampling for *Stygobromus* was conducted in 1985 at 47 sites along the Front Range of Colorado at elevations generally matching the type locale for *S. coloradensis* and *S. pennaki* at the confluence of the North Fork of the South Platte River and its mainstem. A more intensive follow-up study was conducted in 1988 at 30 sites concentrating on the upper South Platte River basin. In the follow-up study both hyporheic and shore zone (phreatic) habitats were sampled using a variety of techniques (Bou-Rouch pump, Karaman-Chappuis technique, and a coarse-mesh wide-mouth net, as appropriate). In the preliminary study *Stygobromus* spp. were collected at only 12 of 47 study sites, with all collections in the vicinity of the type locale. In the follow-up study *Stygobromus* were found at 16 of 30 sites sampled, extending the known distribution in Colorado of *S. coloradensis* and *S. pennaki* well beyond the type locale. The use of multiple sampling techniques was important in locating these organisms. In addition to the amphipod *Stygobromus*, sampling sites in the follow-up study contained a rich interstitial community, including copepods, bathynellids, tardigrades, archiannelids, and ostracods.

*Key words:* *Stygobromus*, Colorado, hyporheic habitat, interstitial organisms.

The subterranean amphipod *Stygobromus* is found in groundwater or groundwater-related habitats throughout the United States (Holsinger 1967, 1974, 1978). There are roughly 100 described species of *Stygobromus*, with at least 30 as yet undescribed species (Holsinger 1978). The majority of recorded species are found in caves (i.e., cave springs, pools, etc.), with other species found in surface springs, wells, seeps, and deep lakes. Two new species, *Stygobromus coloradensis* and *S. pennaki*, were described from the lower North Fork South Platte River, Colorado (Ward 1977), providing the 1st known record of subterranean amphipods from the hyporheic stream gravel environment (Holsinger 1978, 1986). The term subterranean has been expanded in this case to include those species not in direct association with caves, yet still exhibiting the lack of eyes and pigmentation characteristic of cave forms (Holsinger 1986). Prior to the discoveries by Ward (1977), subterranean amphipods had not been reported from Colorado (Pennak and Rosine 1976).

Life histories of *S. coloradensis* and *S. pennaki* are not known. These Colorado species were collected by Ward (1977) only during

April, and in the study by Pennak and Ward (1986), they were also most abundant in spring, generally comprising a mixture of young and mature specimens. *Stygobromus* collected from Lake Tahoe included gravid females (with eggs) from May through December, with immature females, males, and juveniles also present throughout the year (Holsinger 1974). Other western species, which are basically restricted to caves, have been collected at various times throughout the year. What little evidence exists suggests that subterranean amphipods, compared with surface-dwelling amphipods, have longer life spans (>1 yr), produce fewer but larger eggs, and apparently breed continuously at a gradual rate (Holsinger 1986).

The presence of a diverse interstitial community in Colorado streams, in addition to *Stygobromus*, was first described by Pennak and Ward (1986), based on sampling at 1 site on the South Platte River at its confluence with the North Fork, southwest of Denver, Colorado. This work has been followed by additional studies on the South Platte River and other Colorado streams, providing distributional records for a variety of interstitial organisms (Ward and Holsinger 1981, Ward and

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Voelz 1990, 1994, Ward et al. 1992), as well as the discovery of new ostracod, copepod, and bathynellid species (Pennak and Ward 1985a, 1985b, Marmonier and Ward 1990, Reid 1992).

The present study was initiated to determine whether the type of interstitial community described by Pennak and Ward (1986) exists at other locations in central Colorado streams, with particular emphasis on the presence of *Stygobromus*.

#### SAMPLING STRATEGY

A preliminary screening-level study was conducted in the spring and fall of 1985 at over 40 sites along the Front Range of Colorado. This included sites on streams in the

“foothills zone” (Ward and Kondratieff 1992), which resembles the type locale of the North Fork of the South Platte River. Based on results of this preliminary study and Pennak and Ward’s previous study, it appeared that, when found, collections of *Stygobromus* were generally from stream gravels resulting from the decay of Pike’s Peak granite in central Colorado (Ward 1977, Pennak and Ward 1986). Pike’s Peak granite is a fine- to coarse-grained crystalline rock that weathers to a mixture of coarse sands and gravels (Hansen and Crosby 1982, von Guerard 1989). Based on the assumption that these coarse alluvial streambeds may provide suitable habitat for *Stygobromus*, the follow-up study concentrated on sampling

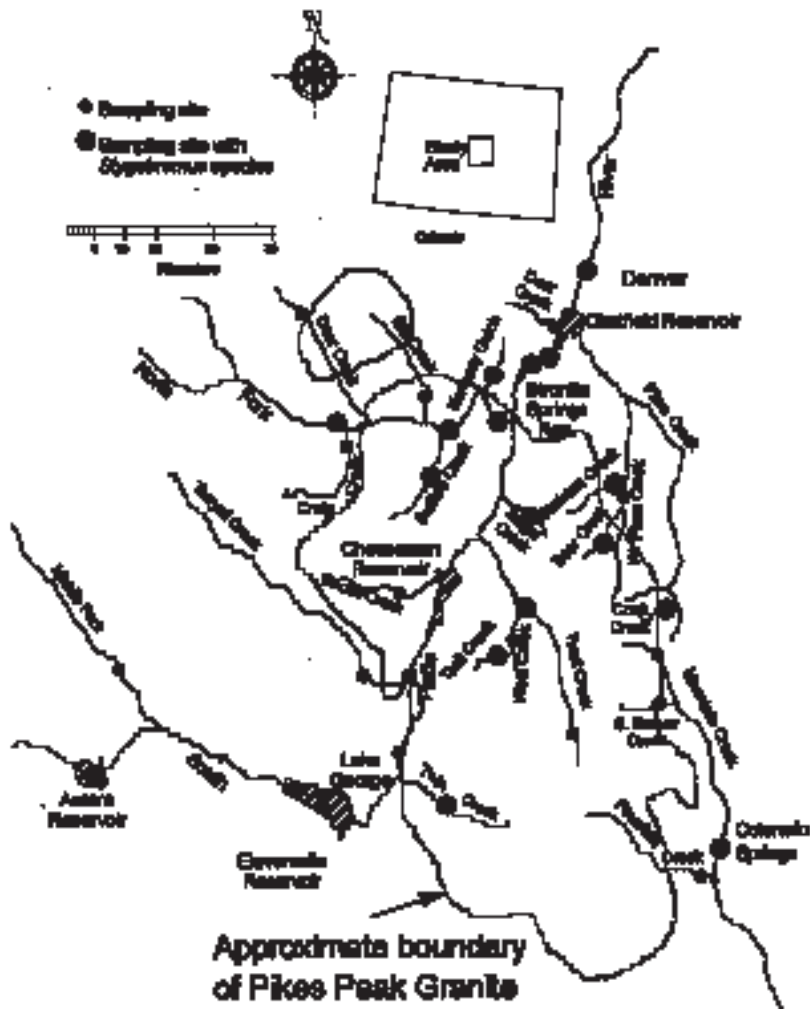


Fig. 1. Sampling locations for the intensive interstitial organisms study in spring 1988.

streams in central Colorado with substrates derived in large part from decomposed Pikes Peak granite (Fig. 1). Sampling was conducted 2 March–28 April 1988 at 30 sites in the South Platte, North Fork South Platte, Plum Creek, and upper Fountain Creek drainages in central Colorado (Fig. 1). *Stygobromus* appear to reach their greatest seasonal abundance in spring (Ward 1977, Pennak and Ward 1986).

During the preliminary study we restricted sampling to the hyporheic zone, which is the interstitial habitat under the streambed (Orghidan 1959). In the later study 2 habitat types were sampled at each stream site. These included (1) the hyporheic zone and (2) the shore zone, which is the boundary between the hyporheic and phreatic environments (Pennak and Ward 1986). These 2 habitats contained the richest assemblage of interstitial organisms in the South Platte River site studied by Pennak and Ward (1986).

#### METHODS

For the initial screening-level study, we sampled each site using a large-mouth net (90 cm wide by 50 cm high) with a relatively coarse mesh size of approximately 240  $\mu\text{m}$ . This net was anchored to the stream bottom with steel rods. The substrate upstream of the

net was worked with a shovel to a depth of 30 cm. Displaced organisms and debris collected in the net were placed in a jar, preserved with 95% ethanol, and returned to the lab. In the lab, while other organisms were collected, samples were sorted only for *Stygobromus*. Although 240- $\mu\text{m}$  mesh is too coarse for collecting most interstitial organisms, it was considered sufficient for the presence/absence determination of these amphipods in this initial study, given the overlapping cohorts.

In the later follow-up study, we used additional sampling techniques. For both the hyporheic and shore zone habitats, 10-L samples were collected with the Bou-Rouch groundwater pump (Bou 1974) from 50-cm depths when possible (Fig. 2). This was the method employed by Pennak and Ward (1986) in their study. The Karaman-Chappuis technique (Delamare Deboutteville 1960, Kolasa et al. 1987) was also used in the shore zone. This method involves digging a shallow pit in the shore zone adjacent to the stream, allowing it to fill with water, and then collecting 10 L (Fig. 2). Following collection of the water, samples from both the Bou-Rouch pump and Karaman-Chappuis techniques were strained through a plankton net (63- $\mu\text{m}$  mesh) and washed into separate jars using 95% ethanol. The large-mouth (240- $\mu\text{m}$  mesh) net described above

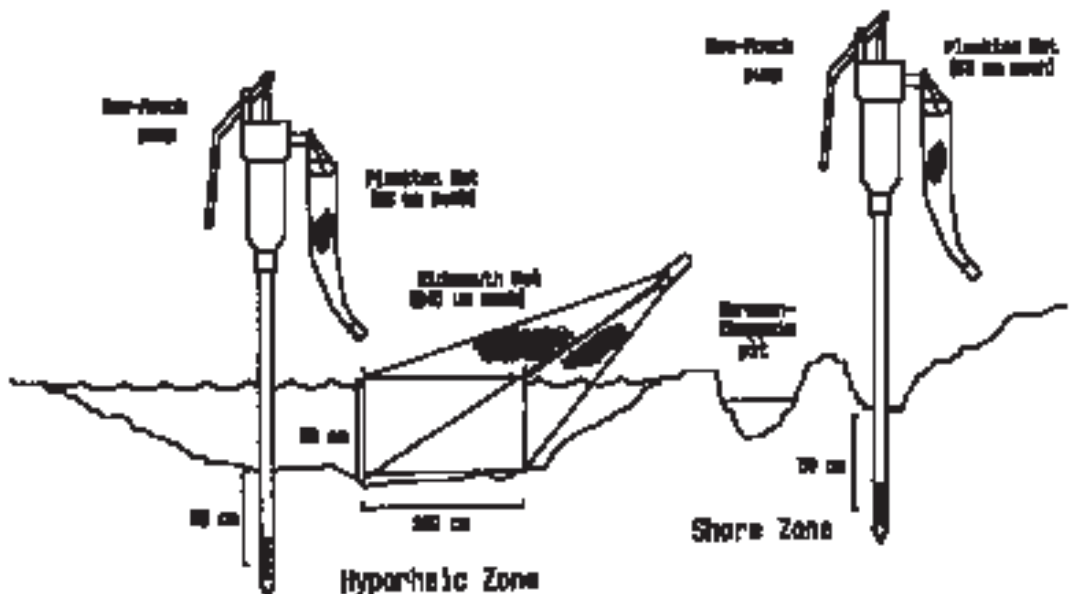


Fig. 2. Schematic representation of sampling methods used for collecting interstitial organisms.

was also used in riffle habitats. In the lab we sorted organisms from all samples from the debris using a dissecting microscope. As before, samples from the large-mouth net were sorted specifically for *Stygobromus* and any larger interstitial organisms, such as cyclopoid copepods. The Bou-Rouch and Karaman-Chappuis samples were sorted for all organisms.

Identification and enumeration of *Stygobromus* specimens were made using keys and descriptions of Holsinger (1967, 1974, 1978) and Ward (1977) and were checked against voucher specimens identified by Dr. John Holsinger, Old Dominion University. Preliminary identifications of other organisms (class or order level) provide additional information on the remainder of the interstitial community.

## RESULTS AND DISCUSSION

### Results of 1985 Preliminary *Stygobromus* Sampling

In this initial study a total of 219 *Stygobromus* were collected at 12 locations (Table 1). These sites are all near the type locale for these species (Ward 1977), as well as subsequent collections (Ward and Voelz 1990, 1994).

No *Stygobromus* were found at the other 35 sites along the Front Range. Generally when *Stygobromus* were found, both *S. coloradensis* and *S. pennaki* were present. The 3rd Colorado species described by Ward (1977), *S. holsingeri*, was not collected in this study. This species was originally collected from a small intermittent spring west of Fort Collins, Colorado. This spring flows only for a few meters during wet periods (Ward 1977). Although several springs similar to this site were sampled in this study, no *Stygobromus* were collected.

Rarely have 2 related *Stygobromus* species been reported occurring in the same location, usually where their respective distributional ranges overlap (Holsinger 1978). However, this is based primarily on the distribution of *Stygobromus* in the eastern United States, where they are largely restricted to caves and springs. Many western species are being collected from hyporheic and alluvial gravels of streams (Ward 1977, Ward and Holsinger 1981, Stanford and Ward 1988). In these habitats it appears to be rare that 2 or more species *do not* occur together. These 2 species do differ in size, with *S. coloradensis* averaging 3 mm long and *S. pennaki* 4 mm. This size difference

TABLE 1. Sampling locations and dates for sites on which *Stygobromus* spp. were collected in the South Platte River basin, spring 1985.

Location	Date sampled	Number of organisms collected	
		<i>Stygobromus coloradensis</i>	<i>Stygobromus pennaki</i>
NORTH FORK			
Between Kennedy Gulch and Dome Rock			
7.0 km upstream of South Platte River	9 April	7	—
200 m upstream of invertebrate site 8	5 April	17	4
100 m upstream of invertebrate site 8	3 April	17	5
Invertebrate site 8, 0.6 km upstream of	26 March	17	2
South Platte River	5 April	3	2
	9 April	9	5
100 m downstream of invertebrate site 8	5 April	2	3
200 m downstream of invertebrate site 8	5 April	2	—
At confluence with South Platte	5 April	17	5
	9 April	18	7
	6 Nov	30	14
SOUTH PLATTE			
Invertebrate site 6, 4.8 km upstream	19 March	—	2
of North Fork			
100 m upstream of North Fork	9 April	2	—
50 m downstream of North Fork	5 April	1	—
200 m downstream of North Fork	9 April	25	2
Invertebrate site 8, 0.75 km downstream	19 March	—	1
of North Fork			
Total collected		167	52

may allow them to utilize different size-related resources, such as food particle size, intragravel space, etc.

Of particular interest in the preliminary study was the occurrence of both species in a November sample. Earlier attempts to collect *Stygobromus* during fall low flows were not successful (Ward 1977). Both the spring and fall *Stygobromus* collections in this preliminary survey yielded a relatively large number of young and mature specimens of both species.

#### Results of 1988 Interstitial Sampling Efforts

**DISTRIBUTION OF *STYGOBROMUS*.**—During the follow-up study 192 *Stygobromus* were collected (Table 2). These again included *S. pennaki* and *S. coloradensis*. In addition, a 3rd, potentially new species was collected that appears to be related to *S. coloradensis* (J.R. Holsinger personal communication). However, too few specimens of this species were collected to allow a true description. Unlike the preliminary study, for these collections *S. pennaki* was generally more abundant than *S. coloradensis* (Table 2).

Prior to this follow-up study, *S. pennaki* and *S. coloradensis* had been reported only from locations on the mainstems of the South Platte River and North Fork South Platte River near their confluence (see above, also Ward 1977, Pennak and Ward 1986). However, in the follow-up study, 1 or both species were collected at 16 of 30 sampling sites, including locations on mainstem and tributary sites in the South Platte, North Fork South Platte, and Plum Creek drainages (Fig. 1, Table 2). The 3rd, undescribed species of *Stygobromus* was found at 2 locations, Monument Creek in the city of Colorado Springs, Colorado, and Twin Creek, directly west of the Monument Creek site near the town of Lake George, Colorado (Fig. 1).

When *Stygobromus* were collected, they were generally found at stream sites either within or downstream of the Pikes Peak granite formation (Fig. 1). This formation generally ranges from just south of Pikes Peak (west of Colorado Springs, Colorado) north to the North Fork of the South Platte River (Tweto 1979). At sites with *Stygobromus*, the substrate largely comprised coarse sands and gravels, consistent with decomposed Pikes Peak granite (von Guerard 1989). Two exceptions were

the collections of *Stygobromus* in Kennedy Gulch and the North Fork upstream of Craig Creek (Fig. 1), just upstream of the reported Pikes Peak granite formation (Tweto 1979). While the presence of stream substrates derived from Pikes Peak granite did not guarantee the presence of these *Stygobromus* species in this study (Fig. 1), these species basically were not found in the absence of such substrate.

***STYGOBROMUS* HABITATS.**—*Stygobromus* were collected primarily from the hyporheic zone of study streams (Table 2), accounting for 75% of total numbers of *Stygobromus* collected during the study. Even when Bou-Rouch samples are compared between habitats, twice as many *Stygobromus* were collected in the hyporheic as in the shore zone. Jackson Creek is the exception: *Stygobromus* were collected only in the shore zone (Table 2). This apparent preference of *Stygobromus* for hyporheic habitats differs markedly from collections of Pennak and Ward (1986) in which *Stygobromus* were found predominantly in the shore zone.

**EFFECTIVENESS OF SAMPLING METHODS.**—Results of the present study also point to the importance of using a variety of collection techniques. While the Bou-Rouch pump is efficient at collecting interstitial organisms (Pennak and Ward 1986, Ward and Voelz 1990, 1994), it has been shown to be less effective than the Karaman-Chappuis technique in certain situations (Strayer 1988). In the present study we used a wide-mouth net and the Karaman-Chappuis technique in addition to the Bou-Rouch pump. In fact, at 5 sites 1 or both *Stygobromus* species were found only in the wide-mesh net and/or Karaman-Chappuis samples (Table 2). These additional methods provided important distribution information that would have been unavailable had only the Bou-Rouch pump been used.

**NOTES ON THE INTERSTITIAL COMMUNITY.**—A diverse interstitial faunal assemblage was collected at all 30 sampling sites (Table 3). These interstitial communities were composed of many of the same groups found by Pennak and Ward (1986) at their site on the South Platte River, including both harpacticoid and cyclopoid copepods, ostracods, bathynellids, archiannelid worms, and mites. In addition, other interstitial organisms not found by Pennak and Ward (1986), but reported in later studies (Ward and Voelz 1990, 1994), such as

TABLE 2. Distribution of *Stygobromus* spp. in central Colorado by location, habitat type, and sampling method, spring 1988 (see also Fig. 1).

Sampling site	Hyporheic		Shore zone		Total collected
	Bou-Rouch (no. 10 L <sup>-1</sup> )	Surface net (no. sample <sup>-1</sup> )	Bou-Rouch (no. 10 L <sup>-1</sup> )	Karaman-Chappuis (no. 10 L <sup>-1</sup> )	
PLUM CREEK DRAINAGE					
Cook Creek					
<i>Stygobromus pennaki</i>	0	42	NA	NA	42
<i>Stygobromus coloradensis</i>	0	18	NA	NA	18
Bear Creek					
<i>S. pennaki</i>	0	3	NA	NA	3
<i>S. coloradensis</i>	6	1	NA	NA	7
Jackson Creek					
<i>S. pennaki</i>	0	0	1	0	1
FOUNTAIN/MONUMENT CREEK DRAINAGE					
Monument Creek-2					
<i>Stygobromus</i> n.sp.?	0	1	0	0	1
SOUTH PLATTE RIVER DRAINAGE					
South Platte River-2					
<i>S. pennaki</i>	4	19	0	0	23
<i>S. coloradensis</i>	6	9	1	0	16
South Platte River-3					
<i>S. coloradensis</i>	1	0	0	0	1
South Platte River-4					
<i>S. pennaki</i>	9	3	4	4	20
<i>S. coloradensis</i>	1	1	1	0	3
Twin Creek					
<i>S. pennaki</i>	0	0	0	2	2
<i>S. coloradensis</i>	0	0	0	2	2
<i>Stygobromus</i> n.sp.?	0	1	0	0	1
Trout Creek-2					
<i>S. pennaki</i>	0	0	1	2	3
<i>S. coloradensis</i>	1	0	4	4	9
Trail Creek					
<i>S. coloradensis</i>	1	0	NA	NA	1
Sugar Creek					
<i>S. pennaki</i>	5	0	0	0	5
<i>S. coloradensis</i>	2	0	2	0	4
NORTH FORK SOUTH PLATTE RIVER DRAINAGE					
North Fork South Platte River-1					
<i>S. pennaki</i>	0	0	0	5	5
<i>S. coloradensis</i>	0	0	0	7	7
North Fork South Platte River-2					
<i>S. pennaki</i>	1	0	2	0	3
North Fork South Platte River-3					
<i>S. pennaki</i>	0	0	3	1	4
<i>S. coloradensis</i>	1	0	1	1	3
Buffalo Creek					
<i>S. pennaki</i>	3	0	0	0	3
Kennedy Gulch					
<i>S. pennaki</i>	2	0	NA	NA	2
<i>S. coloradensis</i>	3	0	NA	NA	3
Total collected					
<i>S. pennaki</i>	24	67	11	14	116
<i>S. coloradensis</i>	22	29	9	14	74
<i>Stygobromus</i> n.sp.?	0	2	0	0	2
All <i>Stygobromus</i> spp.	46	98	20	28	192

TABLE 3. Preliminary distribution of interstitial organisms in central Colorado streams, 1988 (+ = present).

Site	<i>Stygobromus</i> spp.	Harpacticoid/cyclopoid copepods	Ostracods	Bathynellids	Cladocera	Tardigrades	Archannelids	Mites	Nematodes
PLUM CREEK DRAINAGE									
Cook Creek	+	+	+						
West Plum Creek		+	+	+				+	+
Bear Creek	+	+	+					+	
Jackson Creek	+	+	+						
FOUNTAIN/MONUMENT CREEK DRAINAGE									
Monument Creek-1	+	+	+	+	+				
Monument Creek-2		+	+	+					
Beaver Creek		+	+						
Fountain Creek		+	+	+					
SOUTH PLATTE RIVER DRAINAGE									
S. Platte River-1		+	+		+	+	+		+
S. Platte River-2	+	+	+		+	+			+
S. Platte River-3	+	+	+	+	+	+			+
S. Platte River-4	+	+	+	+	+	+	+		+
Middle Fork S. Platte		+	+	+	+	+			
Twin Creek	+	+	+	+	+				
Tarryall Creek		+	+						
Goose Creek		+	+	+					
Trout Creek-1		+	+						
Trout Creek-2	+	+	+		+				
West Creek		+	+						+
Trail Creek	+	+	+						
Sugar Creek	+	+	+	+		+		+	
Deer Creek		+	+		+				
NORTH FORK SOUTH PLATTE DRAINAGE									
N. Fork S. Platte-1	+	+	+						
N. Fork S. Platte-2	+	+	+	+					
N. Fork S. Platte-3	+	+	+	+					
Elk Creek		+	+						
Craig Creek		+	+						
Deer Creek		+	+	+	+				+
Buffalo Creek	+	+	+						
Kennedy Gulch	+	+	+						
Frequency of occurrence (%)	53	100	90	47	40	17	10	10	17



cladocerans and tardigrades, were also collected at a number of sites (Table 3). These organisms have also been reported to be common members of the interstitial community in other studies (Strayer 1988, Danielopol 1989). Copepods were the most common interstitial organism, being found at all 30 sites, with ostracods present at 90% of sites (Table 3). Other crustaceans (amphipods, bathynellids, and cladocerans) were also common, being present at roughly 40–50% of sites. Non-crustacean organisms were encountered much less frequently, often at only 10% of sites.

#### CONCLUSIONS

Prior to the present study, the known range of *S. pennaki* and *S. coloradensis* was basically restricted to 2 adjacent stream reaches in the South Platte River drainage near the confluence of the mainstem and North Fork of the South Platte River (Ward 1977, Pennak and Ward 1986). Other *Stygobromus* species have been reported from springs and small interrupted streams west of Fort Collins, Colorado (Ward and Holsinger 1981), a saline spring in the Piceance Basin in western Colorado (Ward and Holsinger 1981), and the Fraser River near Winter Park (unpublished data). These last 2 are the only records of *Stygobromus* on the west slope of Colorado.

The present study provides 10 new locations for *Stygobromus* in Colorado (Fig. 1), increasing the known range of this genus in the West (Ward and Holsinger 1981, Holsinger 1986). There appeared to be a close association of *S. pennaki* and *S. coloradensis* with alluvial gravels derived from decomposed Pikes Peak granite common to the middle South Platte River basin and adjacent drainages. Results of this study also support findings of Ward and Voelz (1990, 1994) that interstitial communities are widespread in Colorado streams.

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