Subterranean Biology 8: 39-47, 2010 (2011)
doi: 10.3897/subtbiol.8.1230

A new species of the subterranean amphipod crustacean genus *Stygobromus* (Crangonyctidae) from a cave in Nevada, USA

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ABSTRACT

Stygobromus albapinus, a new stygobiotic amphipod crustacean species in the family Crangonyctidae, is described from two pools in Model Cave in Great Basin National Park, White Pine County, Nevada, USA. The type specimens were collected on two different visits to the cave. The new species is assigned to the *hubbsi* group, bringing the number of described species in this group to 45, but many other provisionally recognized species assigned to this group remain undescribed. With exception of a single species from deep wells in southeastern Wisconsin, all other members of the *hubbsi* group are recorded from a wide variety of subterranean groundwater habitats (e.g., caves, springs, wells, etc.) in western North America, west of the Great Plains. Although the taxonomic affinities of *Stygobromus albapinus*, n. sp. need further study, the species does appear to share several important morphological characters with a species from a cave in western Utah located approximately 300 km east-northeast of Model Cave.

Key words: amphipod, *Stygobromus*, subterranean groundwater, stygomorphic, *hubbsi* group

INRODUCTION

The genus *Stygobromus* is comprised exclusively of stygomorphic species (typically lacking eyes and pigment) that inhabit a variety of subterranean groundwater habitats, including cave pools and streams, phreatic water in wells, seeps (hypotelminorheic) and springs, the underflow (hyporheic) of surface streams, and rarely deep lakes (Ward and Holsinger 1981; Holsinger 2009, see also Culver and Pipan 2009). In North America, the genus is represented by 129 described species, although numerous new species have been discovered and are either undergoing description (22 in manuscript by JRH) or are provisionally recognized. In addition, four (and possibly five) species are recorded from Eurasia, bringing the total number of described species in the genus to 133 or 134 (Sidorov et al 2010). In North America more than 50 species are found in the western United States and southwestern Canada (Wang and Holsinger 2001), the majority of which lack sternal gills or processes and have been assigned to the *hubbsi* group (see Holsinger 1974; Wang & Holsinger 2001) primarily on the basis of this diagnostic character. The systematics of the genus in North America are treated in a series of major papers produced over several decades (e.g. Holsinger 1974, 1978, 2009; Wang and Holsinger 2001), along with several others more restricted in scope (Holsinger 1980; Bousfield and Holsinger 1981; Holsinger and Shaw 1986, 1987; Ward 1977). The most recent paper in this series by Sidorov et al (2010) includes descriptions of two new species from Siberia. These papers should be consulted for keys, taxonomic changes, and specific distributional information for the genus.

In the course of a survey of the cave fauna of Great Basin National Park (White Pine County, Nevada), an undescribed *Stygobromus* species of the *hubbsi* group was collected from two different pools in Model Cave in Great Basin National Park, and it is described herein. The description of S. *albapinus* n. sp. increases the number of species assigned to the *hubbsi* group to 45, all but one of which occur in the western Cordillera of North America.

METHODS AND MATERIALS

Specimens were collected directly from cave pools and preserved in 70% ethanol. In the laboratory permanent slide preparations were made by mounting dissected appendages in Faure's mounting medium (a modification of the conventional Hoyer's medium [Upton 1993]) on glass slides. Slide-mounted appendages were photographed using a Leica DM 2500 compound microscope, and stacked digital images were created using Image-Pro Express (version 5.1.1.14, ©2005, Media Cybernetics, Inc.). Images were imported into Adobe Illustrator CS2 (version 12.0.1, ©2005 Adobe Systems, Inc.), where final line drawings were produced. Drawings are of female specimens unless otherwise indicated.

In the description, usage of the descriptive terms "seta" and "spine" are in the original conventional sense and do not agree with terminology suggested by Watling (1989). We use the term "spine" for thick and stiff setae and the term "seta" for thin and flexible structures. No-

Received 6 April 2010; Accepted 12 April 2010

menclature for setal patterns on segment 3 of the mandibular palp follows the system introduced by Stock (1974). The term "defining angle" of the gnathopod propodi refers to the "angle" formed by the end of the palm and beginning of the posterior margin or the point at which the tip of the dactyl closes on the propodus.

SYSTEMATICS

Stygobromus albapinus Taylor & Holsinger**,** sp. nov.
(Figs 1-5)

Material Examined

USA, NEVADA. White Pine County, Great Basin National Park, Model Cave, ♀ holotype and 6 paratypes (1 ♂, 5 ♀♀) collected from pool of water by Gretchen M. Baker, 10 November 2008; 40 paratypes (1 immature ♂, 39 ♀♀) collected by Gretchen and Ben M. Roberts from pool of water (7° C) and sump pool (4° C), 2 February 2009.

The holotype ♀ (4.5 mm) is deposited in the National Museum of Natural History (Smithsonian Institution), USNM 1135494; 5 paratypes are deposited in the Illinois Natural History Survey (INHS) Crustacean Collection (INHS 11447, INHS 11448); remaining paratypes are in the research collection of J. R. Holsinger at Old Dominion University (H-4564, H-4653).

Diagnosis

A relatively small stygobiotic species of the *hubbsi* group distinguished by structure of pereopods 6 and 7, which have relatively long, narrow bases lacking distinct distoposterior lobes; uropod 3, which has a very short ramus bearing 2 small, apical spines; and sub-rectangular-shaped telson with small V-shaped apical notch and 7 to 8 apical spines. This species appears to be morphologically more similar to *S. utahensis,* Wang and Holsinger (2001) from Pole Creek Cave in Duchesne Co., Utah and possibly *S. blinni*, Wang and Holsinger (2001) from Roaring Springs Cave in Coconino Co., Arizona than to any other species in the *hubbsi* group known to date. Largest ♀, 5.0 mm; largest ♂, 3.5 mm

Female (Fig. 1)

Antenna 1 (Fig. 2B) approximately 45% percent length of body, 25 % longer than antenna 2 (Fig. 2C) primary flagellum with 11 segments, most bearing aesthetascs (not shown); accessory flagellum approximately equal in length to 1st flagellar segment. Antenna 2

Fig. 1- *Stygobromus albapinus* new species, (ca. 5.0 mm) female paratype, collected 2 February 2009 in Model Cave, Great Basin National Park, White Pine County, Nevada.

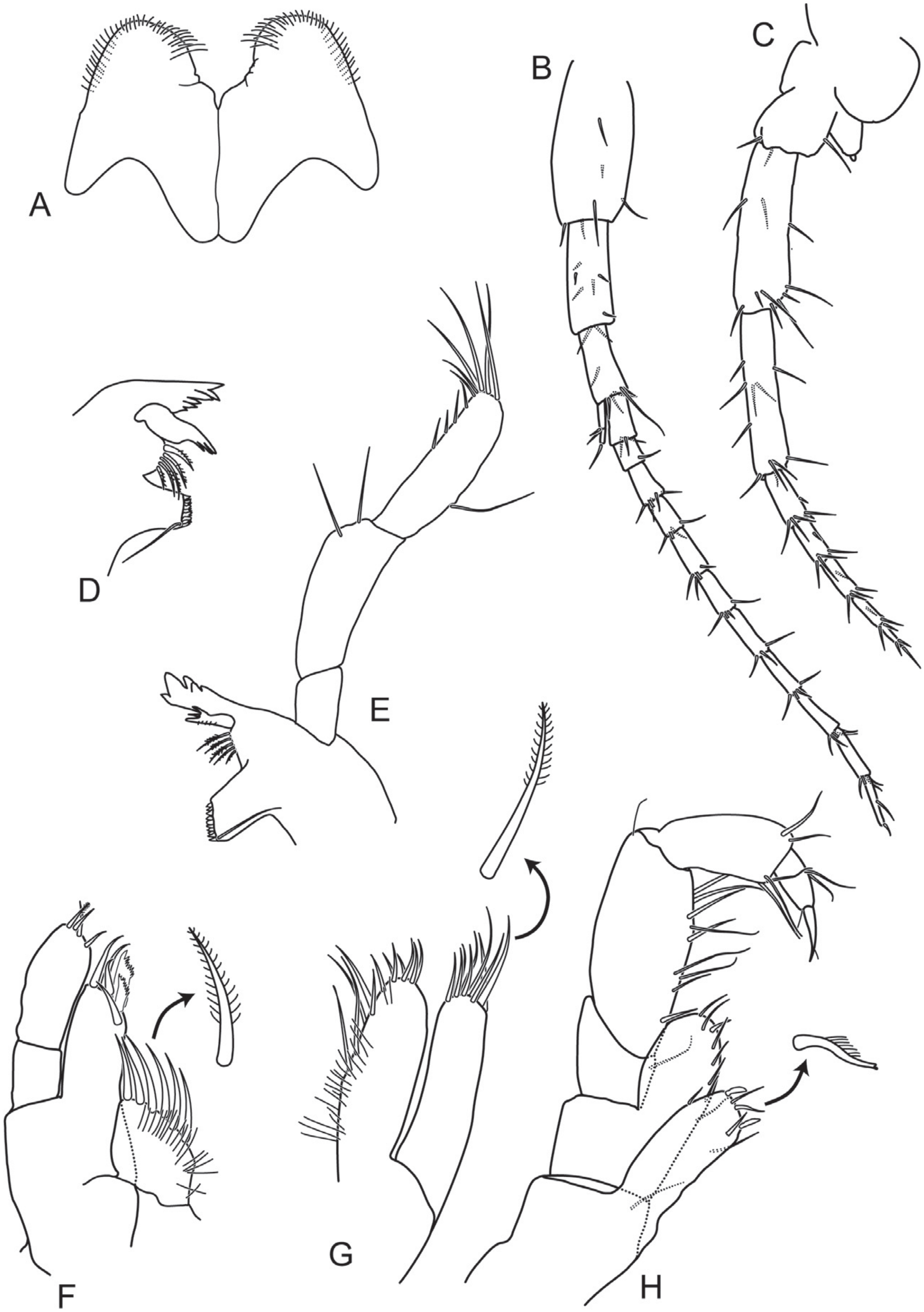

Fig. 2 - *Stygobromus albapinus*, new species, female paratype (5.0 mm), Model Cave, Nevada: A, lower lip; B, antenna 1; C, antenna 2; D, left mandible; E, right mandible; F, maxilla 1; G, maxilla 2; H, maxilliped.

(Fig 2C): peduncular segments approximately equal in length, flagellum with 5 segments. Mandibles (Fig. 2D,E) closely similar but differing in a few minor ways: spine row of both with 5 lightly plumose spines; lacinia mobilis of left with 2 or 3 tiny teeth, that of right apically forked with 3 tiny spines.; incisors normal for genus; mandibles cone shaped, protruding, each with single seta. Palp segments subequal; segment 2 bearing 2 or 3 setae on inner margin distally; segment 3 with 4 long E setae, 5 or 6 shorter D setae and 1 B seta; lacking both A and C setae. Lower Lip: inner lobes present. Maxilla 1 (Fig. 2F): inner plate with 6 apical plumose setae; outer plate with 6 apical spines (2 or 3 with serrated margins); palp with 4 short, apical setae. Maxilla 2 (Fig 2G): inner plate with 4 relatively long naked setae on distal half, followed by approximately 8 shorter setae apically; outer plate with 9 or 10 apical, plumose setae. Maxilliped (Fig. 2H): inner plate with 2 bladelike spines, 1 plumose spine, and 2 naked setae apically; outer plate with 7 or 8 short setae on upper, inner margin; palp segment 2 broader than and more than 2X longer than palp segment 1, inner margin with row of about 8 rather long setae; nail of dactyl (palp segment 4) relatively long and sharply pointed.

Gnathopod 1 (Fig. 3A): propodus smaller than that of gnathopod 2, palm straight and about 25 % longer than posterior margin, armed with double row of about 15 spine teeth in unequal double row; defining angle rounded, with 3 spine teeth on outside, 2 shorter ones on inside; posterior margin lacking setae; 1 superior medial setae, 3 inferior medial setae; dactyl nail short; coxa little broader than deep, ventral margin with 1 seta. Gnathopod 2 (Fig 3B): propodus longer than broad, palm nearly straight, margin irregular, armed with 11 or 12 spine teeth in double row; defining angle with 1 long spine tooth on outside, 2 shorter spine teeth on inside; posterior margin approximately 50 % length of palm, with 2 sets of doubly inserted setae; row of 4 superior medial setae, and 2 inferior medial setae. Coxa of gnathopod 2 slightly broader than deep, margin with 1 seta. Brood plates well developed in sexually mature females (Fig 3C).

Pereopods 3 and 4 (Fig. 4A, B) subequal; coxae subquadrate, slightly broader than deep, reaching about 25 percent of length of basis, ventral margins with 2 setae each. Pereopods 6 and 7 (Figs. 4D, E) subequal in length, 50% length of body, 1.25 X longer than pereopod 5 (Fig. 4C). Bases of pereopods 5-7 relatively narrow, slightly tapering distally but little broader proximally than distally, with few short marginal, distoposterior lobes obsolete; dactyls of pereopods 5-7 relatively long, 30-35 % length of corresponding propodi. Coxal gills (Figs. 4A-D) present on gnathopod 2 and 3-6 (absent from 7). Brood plates (Fig. 3C) relatively long, bearing long distal setae. Sternal gills (processes) absent.

Pleonal plates (Fig.5A): posterior margins weakly convex, each with 1 setule; distoposterior corners weakly rounded; ventral margins weakly convex to nearly straight, plate 3 with single setule. Pleopods (not shown) normal for genus. Urosomites free. Uropod 1 (Fig. 5B): Inner ramus slightly longer than outer ramus, about 78 % length of peduncle, with 9-10 spines; outer ramus with 7 spines; peduncle with 6 spines. Uropod 2 (Fig. 5C): inner ramus markedly longer and broader than outer ramus, nearly as long as peduncle, with 8 or 9 spines; outer ramus with 5 spines; peduncle with 2 spines. Uropod 3 (Fig. 5D): ramus tiny, much smaller than peduncle, bearing 2 tiny spines apically; peduncle at least 4X larger than ramus, with 1 tiny setule. Telson (Fig. 5E) nearly twice as long as broad, apex with distinct V-shaped notch and bearing 7 or 8 spines.

Male

Closely similar to the female but apparently reaching sexual maturity at a slightly smaller size and differing in the morphology of uropod 1 (Fig. 5F) as follows: inner ramus equal in length to outer ramus, about 70 percent length of peduncle, armed with 7or 8 spines; outer ramus with 5 spines; peduncle with 4 small spines on dorsal margin; possession of distinct peduncular process approximately 20 % percent length of peduncle and bearing 5 tiny peg-like spines.

Etymology

The specific epithet *albapinus* is a contraction of *alba* (Latin for white) and *pinus* (Latin for pine), in reference to White Pine County, Nevada—the type-locality and only known population of this species.

Distribution & Ecology

The species is known only from two pools in Model Cave, Great Basin National Park, White Pine County, Nevada, where specimens were collected from both a mud/silt bottom pool with temperature of 7°C and a sump pool with temperature of 4°C. The majority of specimens were collected from the mud/silt bottom pool. Of the 47 specimens sampled, only one is a mature male, suggesting a tendency for parthenogenesis; a trend previously noted for several other species of *Stygobromus* (Culver and Holsinger 1969).

DISCUSSION

The description of *S. albapinus* n. sp. brings the total number of species of *Stygobromus* described from western North America west of the Great Plains to 54, of which 45 are assigned to the *hubbsi* group. Descriptions of 17 new species of *Stygobromus*, and redescription of *S. hubbsi* Shoemaker (1942) from western North America, west of the Great Plains, were provided earlier by Holsinger (1974). This paper was followed more recently by descriptions of an additional 28 new species from the same region by Wang and Holsinger (2001). In addition, there are more than 10 provisionally recognized, undescribed new species of *Stygobromus* from California and

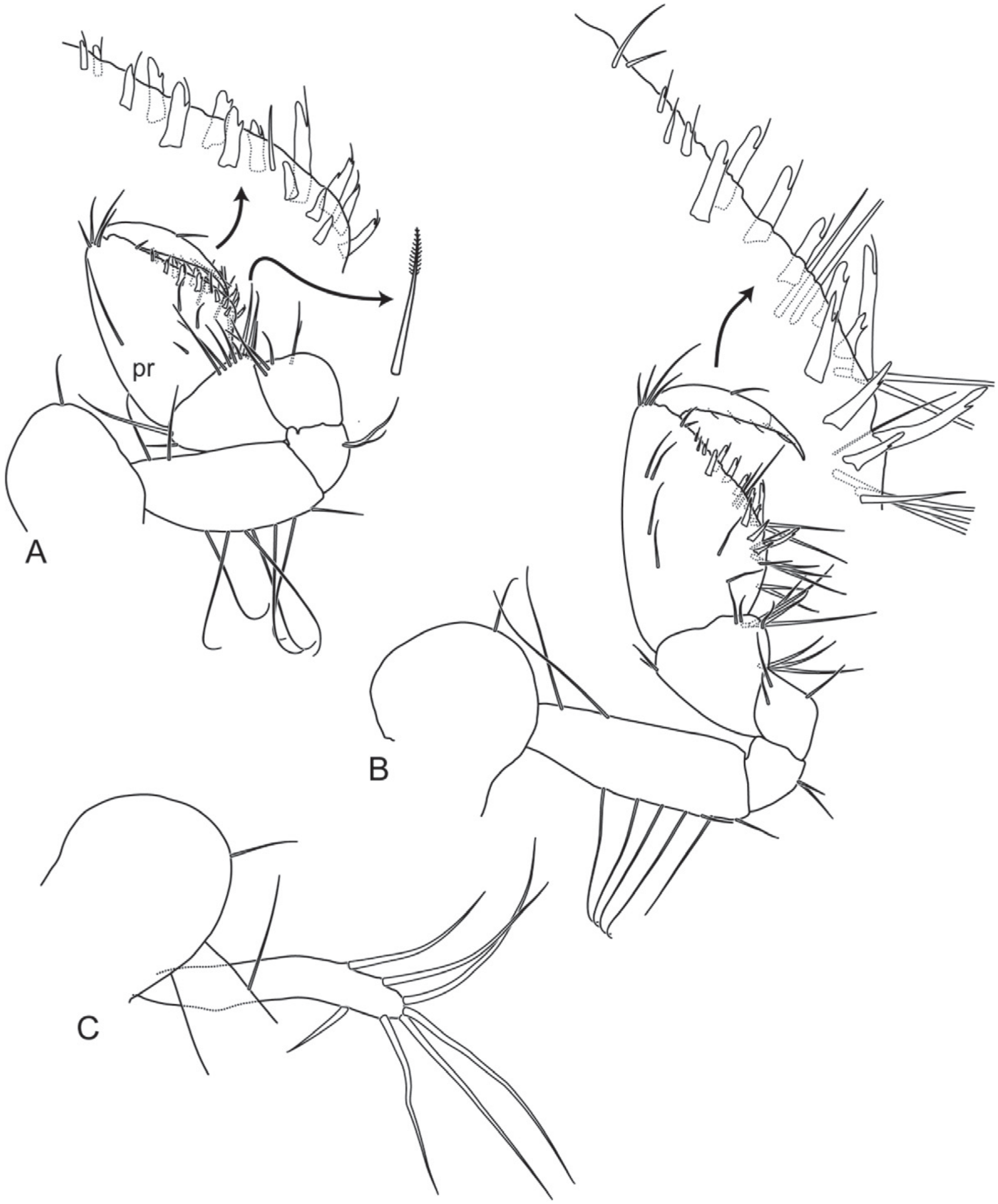

Fig. 3 - *Stygobromus albapinus*, new species, female paratype (5.0 mm), Model Cave, Nevada: A, gnathopod 1 (palm enlarged); B, gnathopod 2 (palm enlarged); C, coxal plate (in part) and setose brood plate of gnathopod 2.

Washington State and several more from southern Alberta, Canada and southeastern Alaska (J. R. Holsinger unpublished data). Most, if not all, of the undescribed species from California and Washington can be assigned to the *hubbsi* group. It is also of biogeographic interest that only one species (*Stygobromus putealis* Holmes) assigned to the *hubbsi* group has been found outside western North America in southeastern Wisconsin (Holsinger 1974; Wang and Holsinger 2001).

Despite relatively close morphological similarities among most of the species, and a preliminary cladistic analysis combined with a list of character states (Wang and Holsinger 2001), the origin, interspecific relationships and biogeography of these species remain in need of further study, especially those assigned to the *hubbsi* group. In addition, given the cryptic habitats and seclusion of groundwater amphipods in western North America, many undescribed

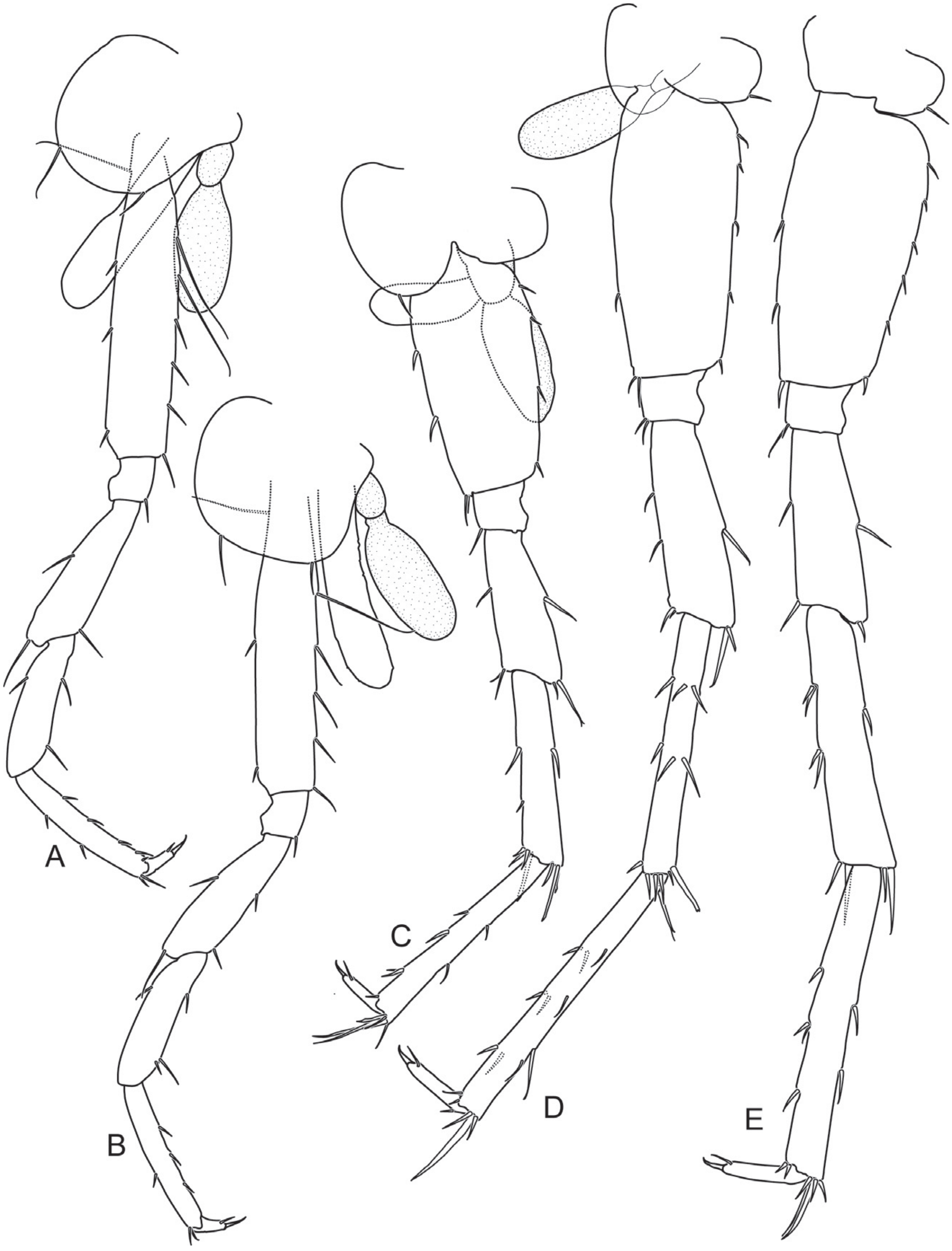

Fig. 4 - *Stygobromus albapinus*, new species, female paratype (5.0 mm), Model Cave, Nevada: A-E, pereopods 3-7, respectively.

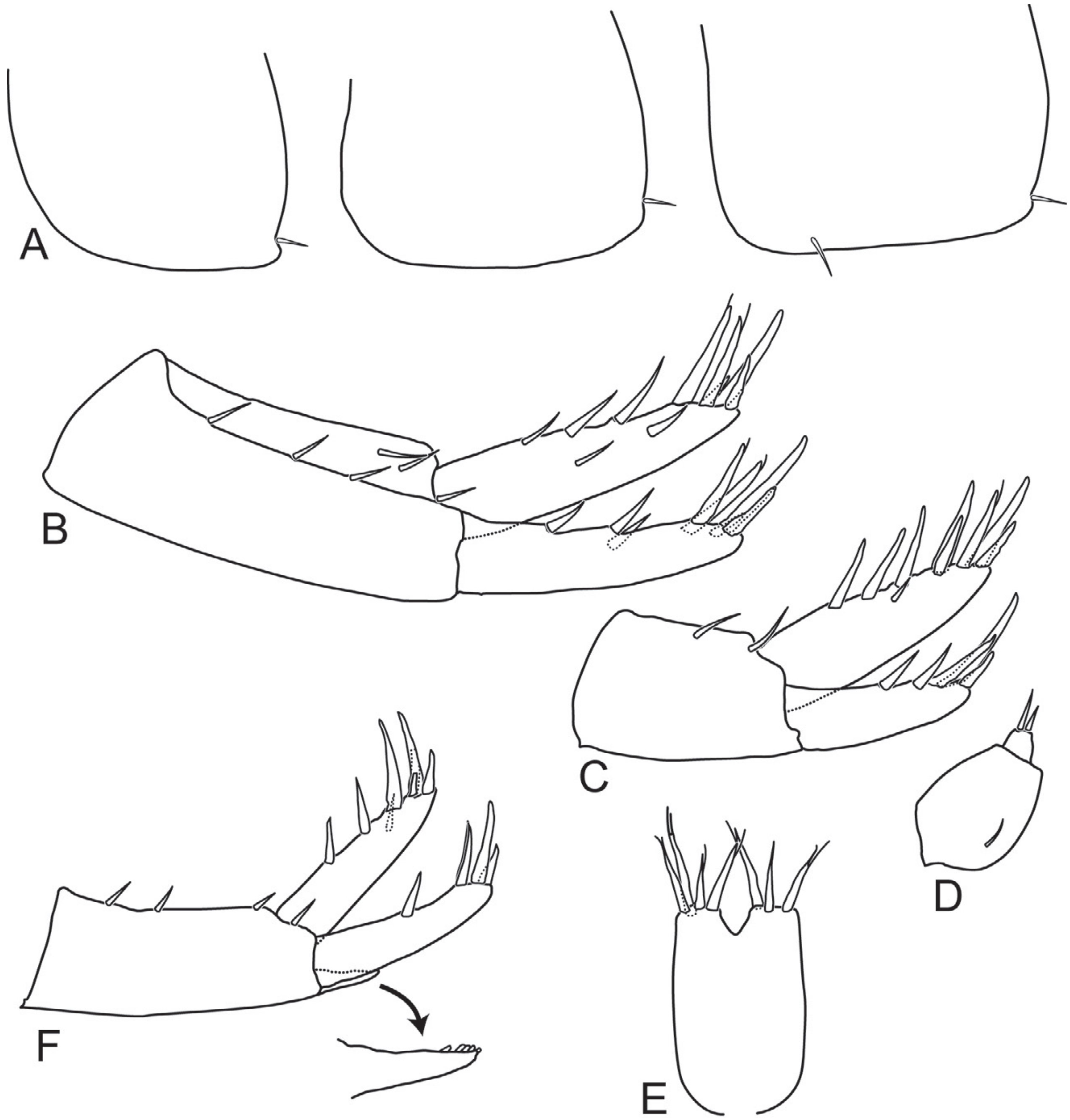

Fig. 5 - *Stygobromus albapinus*, new species, female paratype (5.0 mm), Model Cave, Nevada: A, pleonal plates 1-3; B, uropod 1; C, uropod 2; D, uropod 3; E, telson. Male paratype (3.5 mm), Model Cave: F, uropod 1 (peduncular process enlarged).

species probably remain undiscovered. Thus, future discovery and additional taxonomic study, including molecular analyses, should provide a better idea of the taxonomic and biogeographic relationships among western species.

Wang and Holsinger (2001) suggested that changes in the Pleistocene pluvial lake system of western North America could have affected the distribution of *hubbsi* group species in the Great Basin. Groundwater levels would have been elevated during glacial maxima, followed by disjunctions when groundwater levels were subsequently lowered, and this in turn may have provided the opportunity for speciation in this area. However, it was also noted that the majority of western species occur outside of the areas impacted by the pluvial lakes (Fig. 6), and that the *hubbsi* group is split roughly into a western subgroup that occurs in the Cascades-Sierra Nevada-Coastal Range and an eastern group that occurs in the Rocky Mountains and Colorado Plateau. Between these two groups, the *hubbsi*

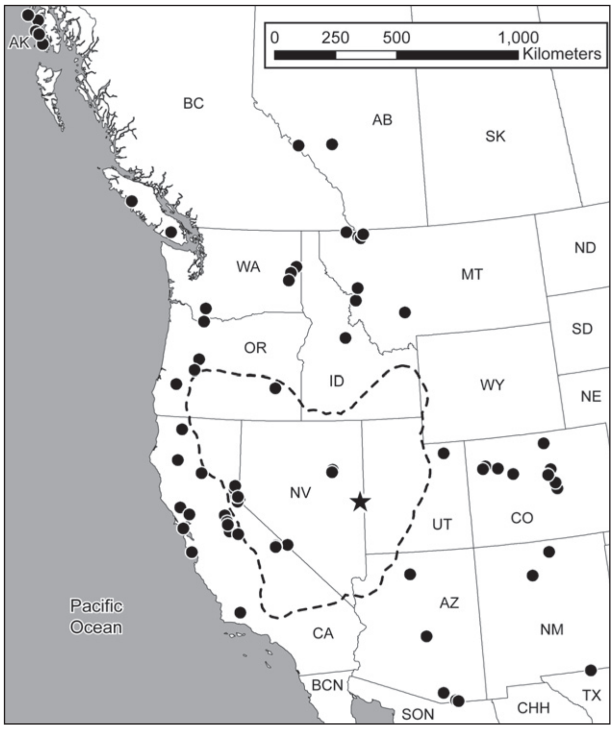

Fig. 6 - Generalized distribution of described species of *Stygobromus* in western North America (closed circles) based in part on Wang & Holsinger (2001), with location of *Stygobromus albapinus* sp. nov. indicated by a star. Dashed line indicates extent of the Great Basin.

group is represented by only seven species that are recorded to date from the Great Basin and Columbia Plateau (Wang and Holsinger 2001). Finally, it is perhaps of interest that none of the non-*hubbsi* group western species occur in the Great Basin or further west (Wang and Holsinger 2001).

What does the occurrence of the new stygobiotic species from Great Basin National Park tell us about the *Stygobromus* fauna of the Great Basin as well as about other cave and groundwater adapted invertebrates? Recent inventories of cave-adapted invertebrate organisms in Great Basin National Park have resulted in the discovery of new species of millipeds and collembolans (Shear 2007, Shear et al 2009; Zeppelini et al 2009), as well as undescribed diplurans and pseudoscorpions. The description of *S. albapinus* n. sp. brings the total number of new invertebrate taxa described from caves in this small area as a result of recent bioinventory work to four. Studies of spring-inhabiting hydrobiid and pleurocerid snails in the Great Basin, many of which occur at one or few sites, have shown that high diversity of springsnail species in the Great Basin is a reflection of their strong association with points of groundwater resurgence and the regional history of aquatic connections (Brown et al 2008). Furthermore, a genetic analysis of amphipods in the epigean genus *Hyalella* (Talitridae) from numerous spring sites in the Great Basin identified a high level of genetic diversity strongly indicating the presence of a multitude of cryptic, undescribed species of *Hyalella* in this region (Witt et al 2006). These findings suggest that the cave and spring faunas of the Great Basin region have not been thoroughly sampled to date. Moreover, sampling methods needed to obtain subterranean amphipods have not to our knowledge been widely implemented in surveys of Nevada's subterranean groundwater faunas. Despite extensive surveys for springsnails and epigean amphipods in the Great Basin, cryptic subterranean groundwater habitats commonly inhabited by *Stygobromus* are not as easily accessed and remain poorly sampled. Utilization of special techniques for sampling small groundwater crustaceans, such as the Bou-Rouch groundwater pump and similar tools (e.g. Leijs et al 2009) for probing relatively deep (~30 cm) into the substrate of spring outlets, may reveal additional undescribed species of *Stygobromus*. A number of factors have to date precluded rigorous sampling of potential groundwater habitats in the Great Basin, perhaps the most important being the marked physical isolation and relatively remote location of aquatic habitats common to Great Basin topography. The occurrence of numerous isolated springs associated with the bases of mountain ranges offers the potential of finding additional populations of *Stygobromus* species within this region. It is likely that the recent discovery of *Stygobromus albapinus* n. sp. will be followed by additional discoveries with further exploration of groundwater habitats in the Great Basin region.

ACKNOWLEDGMENTS

We thank Gretchen M. Baker and Ben M. Roberts (National Park Service, Great Basin National Park) for their contributions to our understanding of subterranean life in Great Basin National Park and, especially, for collecting the specimens of *S. albapinus* n. sp.

REFERENCES

Brown, K.M., B. Lang, K.E. Perez. 2008. The conservation ecology of North American pleurocerid and hydrobiid gastropods. Journal of the North American Benthological Society 27(2): 484-495.

Bousfield, E.L., J.R. Holsinger. 1981. A second new subterranean amphipod crustacean of the genus *Stygobromus* (Crangonyctidae) from Alberta. Canadian Journal of Zoology 59(9): 1827-1830.

Culver, D.C., T. Pipan. 2009. The biology of Caves and Other Subterranean Habitats. Oxford: Oxford University Press.

Culver, D.C., J.R. Holsinger. 1969. Preliminary observations on sex ratios in the subterranean Amphipod genus *Stygonecte*s (Gammaridae). American Midland Naturalist 82 (2): 631-633.

Holsinger, J.R. 1974. Systematics of the subterranean amphipod genus *Stygobromus* (Gammaridae), Part I: Species of the western United States. Smithsonian Contributions to Zoology 160: 1-63.

Holsinger, J.R. 1978. Systematics of the subterranean amphipod genus *Stygobromus* (Crangonyctidae), Part II: Species of the eastern United States. Smithsonian Contributions to Zoology 266: 1-144.

Holsinger, J.R. 1980. *Stygobromus canadensis*, a new subterranean amphipod crustacean (Crangonyctidae) from Canada, with remarks on Wisconsin refugia. Canadian Journal of Zoology 58: 290-297.

Holsinger, J.R. 2009. Three new species of the subterranean amphipod crustacean genus *Stygobromus* (Crangonyctidae) from the District of Columbia, Maryland, and Virginia. Pp. 261-276 *in* S.M. Roble & J.C. Mitchell (eds.), 2009. A lifetime of Contributions to Myriapodology and the Natural History of Virginia. A Festschrift in Honor of Richard Hoffman's 80th Birthday. Virginia Museum of Natural History Special Publication No. 16, Martinsville, VA.

Holsinger, J.R., D.P. Shaw. 1986. A new stygobiont amphipod crustacean (Crangonyctidae, *Stygobromus)* from glaciated karst on Vancouver Island, Canada. Communications 9th International Congress of Speleology, Barcelona, Spain, vol. 2: 98-101.

Holsinger, J.R., D.P. Shaw. 1987. *Stygobromus quatsinensis*, a new amphipod crustacean from caves on Vancouver Island, British Columbia, with remarks on zoogeographic relationships. Canadian Journal of Zoology 65: 2202-2209.

Leijs, R., B. Roudnew, J. Mitchell, B. Humphreys. 2009. A new method for sampling stygofauna from groundwater fed marshlands. Speleobiology Notes 1: 12-13.

Shear, W.A. 2007. Cave millipeds of the United States. V. The genus *Idagona* Buckett & Gardner (Chordeumatida, Conotylidae, Idagoninae). Zootaxa 1463:1-12.

Shear, W.A., S.J. Taylor, J.J. Wynne, J.K. Krejca. 2009. Cave millipeds of the United States. VIII. New genera and species of polydesmidan millipeds from caves in the southwestern United States (Diplopoda, Polydesmida, Macrosternodesmidae). Zootaxa 2151: 47-65.

Sidorov, D.A., J.R. Holsinger, V. Takhteev. 2010. Two new species of the subterranean amphipod genus *Stygobromus* (Amphipoda: Crangonyctidae) from Siberia, with new data on *Stygobromus pusillus* (Martynov) and remarks on morphology and biogeographic relationships. Zootaxa 2478: 41-58

Stock, J.H. 1974. The systematics of certain Ponto-Caspian Gammaridae (Crustacea, Amphipoda). Mitteilungen Hamburg Zoologischen Museum und Institut 70: 75-95.

Upton, M.S. 1993. Aqueous gum-chloral slide mounting media an historical review. Bulletin of Entomological Research 83: 267-274.

Wang, D., J.R. Holsinger. 2001. Systematics of the subterranean amphipod genus *Stygobromus* (Crangonyctidae) in western North America. Amphipacifica 3(2): 39-147.

Ward, J.V. 1977. First records of subterranean amphipods from Colorado with descriptions of three new species of *Stygobromus* (Crangonyctidae). Transactions of the American Microscopical Society 96(4): 452-466.

Ward, J.V., J.R. Holsinger. 1981. Distribution and habitat diversity of subterranean amphipods in the Rocky Mountains of Colorado, U. S. A. International Journal of Speleology 11: 63-70.

Witt, J.D., D.L. Threloff, P.D. Hebert. 2006. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. Molecular Ecology 15(10): 3073-3082.

Zeppelini, D., S.J. Taylor, M.E. Slay. 2009. Cave *Pygmarrhopalites* Vargovitsh, 2009 (Collembola, Symphypleona, Arrhopalitidae) in United States. Zootaxa 2204: 1-18.