

REVIEW

Belgica antarctica (Diptera: Chironomidae): A natural model organism for extreme environments

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Abstract *Belgica antarctica* (Diptera: Chironomidae), a brachypterous midge endemic to the maritime Antarctic, was first described in 1900. Over more than a century of study, a vast amount of information has been compiled on the species (3 750 000 Google search results as of January 10, 2021), encompassing its ecology and biology, life cycle and reproduction, polytene chromosomes, physiology, biochemistry and, increasingly, omics. In 2014, *B. antarctica*'s genome was sequenced, further boosting research. Certain developmental stages can be cultured successfully in the laboratory. Taken together, this wealth of information allows the species to be viewed as a natural model organism for studies of adaptation and function in extreme environments.

Key words *Belgica antarctica*; Chironomidae; stress adaptation; Antarctica

Introduction

Antarctica includes some of the most extreme habitats on Earth (Thomas *et al.*, 2008; Walton, 2013; Convey *et al.*, 2014; Convey, 2017). Besides being one of the harshest environments on the planet with over 99.6% of its area permanently covered by ice and snow, Antarctica is separated from other Southern Hemisphere landmasses by, at minimum, the thousand kilometers of the Drake Passage, and this isolation is further exacerbated by the strong oceanic and atmospheric circulations surrounding the continent. The continent's terrestrial ecosystems are characterized by low species diversity, biomass, and complexity, and reach their greatest extent and complexity in low-altitude coastal regions, especially along the Antarctic Peninsula, where seasonal snowmelt occurs. The Victoria Land Dry Valleys are the largest ice-free

area on the continent but, otherwise, most areas hosting terrestrial life are effectively small and isolated islands surrounded and isolated by inhospitable expanses of ice or cold and seasonally frozen ocean (Bergstrom & Chown, 1999; Convey, 2017). Such features create unique challenges for organismal survival and opportunities to study the adaptations required. However, the question of which species to select and consider as “model organisms” through which to study these biological processes remains open. More generally, “classic” model organisms can be considered nonhuman species that have been widely studied, usually because they are easy to maintain in culture in a laboratory setting, readily reproducing with the potential to generate large sample sizes and practical advantages facilitating experimental studies (Ankeny & Leonelli, 2011; Leonelli & Ankeny, 2013).

Terrestrial ecosystems of the maritime Antarctic region (the Antarctic Peninsula and Scotia Arc archipelagos of the South Shetland, South Orkney, and South Sandwich Islands, and the remote Peter I Øya and Bouvetøya) are generally small in terms of land area and comprise small invertebrates, lower plants, and microorganisms

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(Block *et al.*, 2009; Convey *et al.*, 2014; Convey, 2017). Terrestrial faunal diversity is dominated by mites (>100 species; Pugh, 1993) and springtails (approximately 15 species; Greenslade, 1995). Taxonomic advances continue to take place in Antarctic Collembola (Greenslade, 2018)). Only two insect species—*Belgica antarctica* Jacobs (Diptera: Chironomidae) and *Parochlus steinenii* Gerke (Diptera: Chironomidae)—are native to Antarctica, and both are restricted to parts of the maritime Antarctic (Convey & Block, 1996; Chown & Convey, 2016).

Belgica antarctica is a brachypterous midge endemic to the maritime Antarctic, where it occurs on the South Shetland Islands (Richard *et al.*, 1994) southward along western coastal regions and offshore islands of the Antarctic Peninsula (Janetschek, 1970; Sugg *et al.*, 1983; Allegrucci *et al.*, 2012). In contrast, the winged *P. steinenii* is only found in the maritime Antarctic on the South Shetland Islands, but the species also occurs on sub-Antarctic South Georgia and in southern South America (Convey & Block, 1996; Allegrucci *et al.*, 2006; Contador *et al.*, 2020).

In addition to these two native midges, two nonnative and possibly invasive species are present at specific locations in the maritime Antarctic—*Eretmoptera murphyi* (Diptera: Chironomidae) (Worland, 2010; Hughes *et al.*, 2013) and *Trichocera maculipennis* (Diptera: Trichoceridae) (Chown & Convey, 2016). To date, the latter two species only occur on their islands of introduction, Signy Island (South Orkney Islands) and King George Island (South Shetland Islands), respectively. However, their distribution on both islands has expanded considerably since their introduction (Volonterio *et al.*, 2013; Potocka & Krzeminska, 2018; Bartlett *et al.*, 2020), and there is now a substantial risk of both species expanding within the maritime Antarctic (Perterra *et al.*, 2019; Remedios-De León *et al.*, 2021). The depauperate insect diversity of the Antarctic stands in contrast to that of the Arctic where, by 1990, over 1650 insect species were known to occur (Convey & Block, 1996). Even on the High Arctic Svalbard archipelago (which lies at 78–80°N) at least 337 insect species are known (Coulson *et al.*, 2014).

Adaptations to extreme habitats can be studied using multiple approaches, from ecology and life history to the multiple “omics” disciplines. Genetic adaptive mechanisms are a particular subject of focus in studies of the model laboratory insect *Drosophila melanogaster* (Flatt, 2020), but other Diptera are being studied increasingly (Wiegmann & Richards, 2018; Contador *et al.*, 2020). Among Antarctic invertebrates, *B. antarctica* is often chosen in studies of biochemical and physiological adaptation mechanisms and gene expression in se-

vere environments (Lee & Denlinger, 2010; Michaud & Denlinger, 2010; Teets & Denlinger, 2014; Chown & Convey, 2016; Finch *et al.*, 2020), along with a range of springtails, mites, nematodes, and tardigrades (Kaczmarek *et al.*, 2018; Caruso *et al.*, 2019; Carapelli *et al.*, 2020; Gañan *et al.*, 2021)

Species of Chironomidae have long been used in studies of the morphological structures and mechanisms underlying different biological processes (Balbiani, 1881), with a history extending as far as that of *D. melanogaster*. Since its original taxonomic description (Jacobs, 1900), *B. antarctica* has been a focus of research attention as methodologies have developed, up to the innovative approaches of genome sequencing and single-gene expression studies of the present day (Wirth & Gressitt, 1967; Strong, 1967; Janetschek, 1970; Michaud *et al.*, 2008; Li *et al.*, 2009; Goto *et al.*, 2011; Teets *et al.*, 2012b; Kelley *et al.*, 2014; Finch *et al.*, 2020).

The western Antarctic Peninsula and Scotia Arc region experienced rapid warming in the second half of the twentieth century, a trend which, although paused in the early years of the current century, is predicted to resume (Turner *et al.*, 2009; Convey & Peck, 2019). This regional climate change has led to increased thawing of ice and snow, longer and warmer active summer seasons, and increased area of ice-free ground available for the development of terrestrial habitat—trends predicted to continue throughout this century (Lee *et al.*, 2017; Convey & Peck, 2019; Siegert *et al.*, 2019). Native, including endemic, terrestrial invertebrate species appear to be typified by considerable life history and ecophysiological flexibility. This may be an evolutionary consequence of the considerable magnitude of both short- and long-term variation in their natural microenvironmental conditions, such that in many instances (and in the absence of invading nonnative competitors) they appear likely to be able to take advantage of ameliorated conditions and to “benefit” in terms of distribution, biomass, and population growth (Convey, 1996, 2011; Convey & Peck, 2019; Siegert *et al.*, 2019). Species such as *B. antarctica* are therefore of particular interest in terms of their ability to adapt and respond to changing environmental conditions, potentially being key indicators of the ecosystem consequences of global, human-induced, environmental change.

In this review, we first present a synthesis and overview of the wealth of data across multiple disciplines that have been generated in studies of *B. antarctica* relative to most other Antarctic species. We identify key advantages offered by this body of research. We then propose and argue that *B. antarctica* be viewed as an ideal model organism for field research on adaptation mechanisms and survival strategies in response to the multiple challenges

presented by the extreme environment of Antarctica and, by extension, other regions with analogous conditions.

Ecology and distribution

The Belgian Antarctic Expedition (often referred to as the “Belgica expedition”) on the steam-yacht *Belgica* in 1897–1899, organized by the Belgian Government, was the first of a series of scientific expeditions to explore the Antarctic Peninsula region (W.E.P., 1905). During this expedition, Emile G. Racovitza collected an apterous midge and its larvae from locations along the west coast of the Antarctic Peninsula. Jacobs (1900) assigned this midge to the genus *Belgica* (Chironomidae, Orthocladiinae, Diptera) (Peckham, 1971). It is the largest native terrestrial invertebrate resident in Antarctica.

Two species are currently recognized in the genus *Belgica*: *B. antarctica* Jacobs (1900), endemic to the maritime Antarctic, and *B. albipes* Séguy *et al.* (1965), endemic to the sub-Antarctic Îles Crozet in the Indian Ocean sector of the Southern Ocean. The latter species was initially assigned to different genus before being transferred into the genus *Belgica* (Séguy, 1965; Serra-Tosio, 1982). Unlike *B. antarctica*, the biology of *B. albipes* is almost completely unknown (Hullé *et al.*, 2018). Historically, at least one other species has been assigned to the genus *Belgica*—an intertidal chironomid, originally described as *B. magellanica* by Jacobs (1900) from a single location in the Beagle Channel in the Magellanic sub-Antarctic region of southern South America, that was only recently rediscovered and studied (Simões *et al.*, 2020). This species is now known as *Telmatogeton magellanicus* (Jacobs, 1900), having initially been transferred from *Belgica* to *Jacobsiella* and then *Halirytus*, with these genera now considered junior synonyms of *Belgica*. This species now has an increasing number of recorded occurrence locations centered along the Beagle Channel but also including the Falkland Islands (Nondula *et al.*, 2004; Simões *et al.*, 2020).

Another possible relative of *B. antarctica*, described in the literature as its “sister species” (Cranston, 1985), is *Eretmoptera murphyi* Schaeffer, 1914. Based on analyses of two genetic markers, Allegrucci *et al.* (2012) suggested that *E. murphyi* is sufficiently close to *B. antarctica* and *B. albipes* to be properly reclassified in the genus *Belgica*. However, this proposal has yet to be formally addressed, requiring further morphological and molecular studies. Palaeoendemic to sub-Antarctic South Georgia, this species is thought to have been introduced to Signy Island in the maritime Antarctic South Orkney Islands in 1967 during transplantation studies of vascu-

lar plant species from the Falkland Islands and South Georgia to assess their potential for survival under more extreme conditions (Edwards & Greene, 1973; Edwards, 1980; Convey & Block, 1996). Although such experiments would now not be permitted under the Protocol on Environmental Protection to the Antarctic Treaty, the transplants included plants and soil from within *E. murphyi*’s known range on South Georgia, with no biosecurity precautions required or taken to prevent inadvertent movement of associated terrestrial invertebrates. No invertebrates were searched for or noted at the time of the transplants, and it was not until the early 1980s that the fly was first reported on Signy Island (Block *et al.*, 1984). However, live larvae of *E. murphyi* have certainly been transported inadvertently into Antarctica from South Georgia on a subsequent occasion, when over 100 kg of soil that had not been properly cleaned from construction vehicles before relocation to Rothera Station on Adelaide Island was found to contain the species, as well as a range of other invertebrates, plants, and microbes (Hughes *et al.*, 2010). It is also possible that the species was introduced to Signy Island earlier, during the 1930s operation of a small whaling station at the same location as the current research station, because this also involved the movement of vessels and cargo from South Georgia (Convey & Block, 1996).

Belgica antarctica occurs along the length of the Antarctic Peninsula and South Shetland Islands (Peckham *et al.*, 1971; Convey & Block, 1996; Allegrucci *et al.*, 2006; Harada *et al.*, 2014), but it does not occur on the South Orkney Islands. Its northern-most record is from Elephant Island (61°10’S, 55°14’W), while the known southern boundary of its range has extended as more of that region has been sampled (Greene *et al.*, 1967; Usher & Edwards, 1984). The most southern published records are from Red Rock Ridge (Fallières Coast) (68°17’S, 67°12’W) and the Refuge Islands (Marguerite Bay, 68°21’S, 67°08’W), with speculation that the species also occurs on the Terra Firma Islands (68°43’S) (Usher & Edwards, 1984). There is an unpublished record of a single larva extracted from a moss sample obtained from a well-vegetated, unnamed peninsula in Lazarev Bay, northern Alexander Island, and this is currently the species’ “furthest south” occurrence at 69°22.0’S 71°50.7’W (P. Convey, unpublished data); this is also the furthest south known occurrence of the two native Antarctic flowering plants (Convey *et al.*, 2011). The occurrence of *B. antarctica* is facilitated by the presence of moist vegetated habitats such as algal mats, bryophytes, and flowering plants (Convey & Block, 1996; Ochyra *et al.*, 2008; Parnikoza & Kozeretka, 2020). Larvae have been reported in association with the nitrophilous foliose

alga *Prasiola crispa*, in mosses and in the rhizosphere of the grass *Deschampsia antarctica* (Peckham, 1971), as well as in cyanobacterial mats in seasonal streams (Richard *et al.*, 1994) and shallow lakes associated with green algae (Wirth & Gressitt, 1967; Harada *et al.*, 2014). The highest larval densities are reported from mosses (Gressitt, 1967; Potts *et al.*, 2020). Adult flies are active on the ground, rocks, and plant surfaces, particularly during warm, bright weather (Convey & Block, 1996). After mating, the females lay their eggs as a single batch in moist locations (Strong, 1967; Edwards & Baust, 1981).

The larvae are thought to be nonselective feeders (Baust & Edwards, 1979), consuming decaying vegetation, algae, fungi, and other microorganisms (Strong, 1967; Sugg *et al.*, 1983; Usher & Edwards, 1984; Rinehart *et al.*, 2006; Harada *et al.*, 2014). The activation of stress tolerance strategies in larvae (such as cryoprotective dehydration) and exposure of adults to dehydration have been suggested as possibly reducing adult fertility (Finch *et al.*, 2020). In other insect species, reproductive output can also be reduced because of nutrient deficiency, exposure to xenobiotics, unfavorable temperatures, and other stress factors (Aguila *et al.*, 2007, 2013; Vogt *et al.*, 2007; Rosa & Saastamoinen, 2017; Finch *et al.*, 2020).

Life cycle

Compared with many other Diptera, *B. antarctica* has an extended biennial life cycle (Sugg *et al.*, 1983; Usher & Edwards, 1984; Convey & Block, 1996; Rinehart *et al.*, 2006). Life cycle extension in the extreme conditions of the Antarctic compared with those of phylogenetically related species from tropical and temperate latitudes is a commonly reported feature in life history studies of Antarctic (and Arctic) invertebrates (Convey, 1996; Søvik, 2004a,b; Rinehart *et al.*, 2006). Such extension may simply be a consequence of reduced energy availability and not in itself an evolutionary adaptation. However, a feature often associated with it is the ability of multiple developmental stages to survive over winter, which is generally unusual in invertebrates and suggests the evolutionary loss of a defined single or low number of overwintering stages (Convey, 1996).

Like all other Diptera, *B. antarctica* has complete metamorphosis. The four stages are the egg, the larva, the pupa, and the adult (Convey & Block, 1996; Harada *et al.*, 2014; Finch *et al.*, 2020). Egg development takes 16 days at 4°C (Harada *et al.*, 2014). The larval stage is the longest, lasting almost 2 years, and includes four instars (Usher & Edwards, 1984), as is typical of other Chironomidae (Bartlett *et al.*, 2019b; Finch *et al.*, 2020). Wirth



Fig. 1 Mating pair of *Belgica antarctica* (photo R. E. Lee).

and Gressitt (1967) suggested that the pupae can survive the winter, while other studies have reported that larvae pupate early in the austral summer in late November and that the adults emerge approximately 1 month later and can be observed from late December through to late January (Martin, 1962), as is also the case in *E. murphyi* (Bartlett *et al.*, 2019b). The nonfeeding adults survive only 1–2 weeks after their summer emergence (they are found from late December to March) (Sugg *et al.*, 1983; Usher & Edwards, 1984; Rinehart *et al.*, 2006; Finch *et al.*, 2020). The apparent inconsistencies in published descriptions of adult phenology may result from observations being made at different locations and/or the influence of intra- and interannual climatic variation.

The adults (Fig. 1) are dark in color—from deep black to dark reddish-brown, with lighter heads and antennae—and they exhibit sexual dimorphism. The females are somewhat larger than the males, measuring 1.5–3.2 mm in length compared with the 1.6- to 2.5-mm-long males. The antennae of females are shorter. Males have more facets per eye (35–44 compared with the female's

29–33) (Wirth & Gressitt, 1967; Meyer-Rochow & Reid, 1994) and narrower tapering abdomens bearing more setae, which are also thicker than those of the female. The genitalia were described by Wirth and Gressitt (1967). In the laboratory, the lifespan of the male is one-third longer than that of the female. Most females die shortly after laying their only egg batch because the process damages their abdomens (Harada *et al.*, 2014). *Eretmoptera murphyi* similarly lays a single gelatinous egg batch in the form of a ball, which is thought to provide some desiccation resistance (Convey, 1992; Bartlett *et al.*, 2019a). In field samples, adult sex ratios that are substantially skewed toward males have been reported (Wirth & Gressitt, 1967; Sugg *et al.*, 1983), although this has not been noted at pupal emergence in the laboratory. Copulation typically occurs immediately after emergence, and tends to occur in clusters of insects (Edwards & Baust, 1981). A spermatophore containing sperm and other substances (likely products of the accessory gland) is transferred as a bundle to the female (Finch *et al.*, 2020). Several males typically attempt to mate with a single female, and males can mate more than once (Strong *et al.*, 1967; Edwards & Baust, 1981). Females lay their egg batch 1 day after copulation in sheltered, moist sites. The egg batch typically contains 30–60 eggs, fewer than in the batches of *E. murphyi* (Convey, 1992; Harada *et al.*, 2014; Bartlett *et al.*, 2019a). The eggs are coated with a hygroscopic gel, which may provide a food substrate for the developing larvae, that is secreted by the female accessory gland during oviposition (Edwards & Baust, 1981; Harada *et al.*, 2014). Analysis of the proteins in the gel has revealed three main components: vitellogenin, larval serum protein, and apolipoprotein (Finch *et al.*, 2020). The gel also protects the eggs from dehydration and acts as a thermal buffer, preventing overheating (Finch *et al.*, 2020). At 6–7°C, virgin females will lay unfertilized eggs 5–7 days after hatching. These eggs do not develop, suggesting the species lacks parthenogenesis, unlike *E. murphyi* (Edwards & Baust, 1981; Cranston, 1985; Convey & Block, 1996; Bartlett *et al.*, 2019b).

While long-term reproducing cultures of *B. antarctica* have not been established under laboratory conditions, larvae have reliably been maintained over periods of several months. Benoit *et al.* (2007) housed the midges in individual mesh-covered cages, while Harada *et al.* (2014) transported the larvae from Antarctica and obtained the adult midges *in vitro*. Finch *et al.* (2020) managed to mate captive imagos, which laid both fertilized and unfertilized eggs and observe the early stages of larval development.

Larvae are normally collected from natural habitats. Larvae in organic debris (rocks, soil, detritus, moss, and algae) are typically transported to the laboratory and kept



Fig. 2 Fourth-instar larvae of *Belgica antarctica* (photo British Antarctic Survey).

in airtight boxes under an 18-hour light to 6-hour dark cycle. The substrate is sprayed with water once every 2 days to maintain high humidity. Imagos that eclose are transferred immediately into airtight plastic containers containing damp paper towel under the same light/dark regime, where the females lay eggs. During transport and maintenance in the laboratory, the temperature is kept at 4°C (Benoit *et al.*, 2007; Harada *et al.*, 2014; Finch *et al.*, 2020).

Under laboratory conditions, egg development takes over 2 weeks, and only 15% of the eggs hatch. The chorion is translucent, making it possible to distinguish the eyes and head of an embryo at the end of its development. By the time of hatching, the larva can perform peristaltic movements (Harada *et al.*, 2014). The larval stage lasts up to 2 years and includes four instars, as is typical of Chironomidae, including the related *E. murphyi* (Sugg *et al.*, 1983; Usher & Edwards, 1984; Bartlett *et al.*, 2019b). Larvae of different instars differ in color and size, and the fourth-instar larvae (Fig. 2) can reach up to 5 mm in length (Wirth & Gressitt, 1967). Female larvae are significantly larger than male larvae (Atchley & Hilburn, 1979). The larva has three thoracic and eight



Fig. 3 Pupa of *Belgica antarctica* (40× magnification, 600× 600 DPI). Photo taken using an Olympus E-410 digital camera (China) attached to an Olympus SZX12 microscope (China). Sets of 10 images were taken and assembled to deep-focused images using Helicon Focus 6 and edited in Adobe Photoshop CS6.

abdominal segments, with more intense coloration on the dorsal than the ventral side (Usher & Edwards, 1984). The sex of the fourth-instar larva can be determined by the genital anlage and by the presence or absence of heterozygotes for a sex-linked inversion, with Inversion D always being heterozygous in males (Atchley & Davis, 1979). After the larva hatches, it remains in the gel for a short time before leaving and starting to feed on the substrate. The first-instar larvae are very small and likely feed on minute particles of detritus, yeast, and bacteria, but later instars consume other fungi and algae. Dense populations of *B. antarctica* are often found at sites where moss formations are enriched with waste from seabirds or seals, which are also rich in algae and microorganisms. All four instars can overwinter (Sugg *et al.*, 1983).

The pupa (Fig. 3) is somewhat smaller than the final-instar larva. It has a whitish conical head. The folded legs are positioned near the head. The abdomen gradually tapers, and the genitalia are free and projecting. A thoracic respiratory organ is not present. At the completion of this stage, the eyes and wings become visible (with the wings being considerably larger than those of the adult) (Wirth & Gressitt, 1967). In the field, males start pupating somewhat earlier (1–3 days) than females (Sugg *et al.*, 1983), although, as noted above, both sexes continue emerging together for several weeks during the summer. Together with the females' shorter lifespan, such sex differences may underlie the skewed sex ratios reported in some field studies.

The details of the life cycle can be influenced by variations in local conditions (Sugg *et al.*, 1983). For instance, spring 2006 initiated abnormally early, which led to an early adult emergence (Schulte *et al.*, 2008). In laboratory experiments examining the effect of temperature on embryonic developmental rates, the developmental period was shortened from 16 to 10 days as temperature

was increased from 4°C to 13°C (Harada *et al.*, 2014). However, much remains to be learnt of the influence of environmental parameters, and changes therein, on the life cycle of *B. antarctica*.

Under laboratory conditions, pairwise comparisons of gene expression between larvae and adult males and females of *B. antarctica* have shown that 862 genes in larvae, 392 genes in males, and 1825 genes in females are uniquely upregulated (Finch *et al.*, 2020). In females, most of these genes are involved in modulation of DNA repair, cell cycle regulation, organization of chromatin, metabolism of cyclic organic cell compounds, and primary metabolism. In males, the genes relate to carbon acid synthesis, anion transport, and immune system development. In larvae, the metabolism of aminoglycans and reduction–oxidation processes were amongst the most noticeable (Finch *et al.*, 2020). An analysis of tissue-specific gene expression has also been conducted for larvae, males, and females, with significant differences being reported between somatic and reproductive tissues, even within one sex and one ontogenetic stage. In the accessory glands of the female reproductive system, 20 genes regulating the activity and response of ionic channels to stimuli, binding of phosphatase, and glycosylation showed distinctive expression levels. In the accessory glands of the male reproductive system, 25 genes regulating membrane composition and metalloproteinase activity were reported (Finch *et al.*, 2020). Notably, amongst the genes identified in that study that showed sex, stage, and tissue specificity in their expression, 14 were transcription factors. One had binding sites specific to genes uniquely upregulated in males, while the other 13 were specific to females (five of these were upregulated in females both at the whole-body level and specifically in the accessory glands) (Finch *et al.*, 2020). Based on these data, the authors concluded that the transcription factors identified are crucial for the development and functioning of the reproductive system in female *B. antarctica*. They include *FOX1* and the *MAD1* homolog protein; in *Drosophila*, *MAD* is part of the *Dpp*-signal pathway that influences the polarization of the egg during early embryonic development (Finch *et al.*, 2020).

Differential gene expression has also been analyzed in the females, males, and larvae of *B. antarctica* and compared with eight other species of Diptera, including four species of Chironomidae and four species of *Anopheles* (Finch *et al.*, 2020). The greatest similarity in gene expression levels was apparent in the females. Across all species, over 1267 genes were upregulated. Functionally, the products of these genes were linked to DNA, RNA, nuclease activity, RNA transport, and nuclear protein transport, regulation of the cell cycle,

organization of organelles and chromosomes, and RNA processing. Females of *B. antarctica* showed no unique upregulated genes, which was also the case with males of the studied taxa. In all cases, the upregulated genes were related to the catabolism of alpha-amino acids, anion transport, and transmembrane transport of carbon acids. The larvae of all species studied upregulated the same genes, which are primarily related to chitin metabolism and cytochrome *P450*-dependent binding of iron ions. Only two genes unique to *B. antarctica* larvae were identified—one coding *polyubiquitin B* and the other unknown (Finch *et al.*, 2020).

The life cycle and physiology of *B. antarctica* are clearly well-adapted to enable survival in the harsh Antarctic environment, while gene expression profiles for both larvae and adults show few or no distinct features compared with other Diptera. This suggests that, despite its unusually extended life cycle and exceptional geographical distribution, the species still has potential for use as a model to study adaptation mechanisms to extreme and variable environments more generally.

Cytogenetic traits

The karyotype of *B. antarctica* is $2n = 6$ (Atchley & Davis, 1979), while in other Chironomidae, the typical set is $2n = 8$ (Martin *et al.*, 1974). However, this chromosome set is well represented in other members of the subfamily Orthocladiinae and is seen as the basic karyotype (Bauer & Beermann, 1952; Atchley & Davis, 1979). *Belgica antarctica* and many other Chironomidae also have polytene chromosomes in the larval salivary glands. Unfortunately, there are no data available for other developmental stages, organs, and tissues of the species, although the structures are known from other chironomids (Zhimulev, 1996). The polytene chromosomes of *B. antarctica* are not large, which complicates their analysis (Atchley & Davis, 1979). Chromosome I is the shortest and has a large Balbiani ring a quarter of the chromosome's length away from one of its ends and a submedial puff with a nucleolus organizer region (NOR). Chromosome II has a medial puff with an NOR and a dark subterminal band. Chromosome III is the longest and has a subterminal puff with an NOR and, unlike chromosome II, lacks clear subterminal dark bands (Atchley & Davis, 1979).

Early studies examined chromosome polymorphism in *B. antarctica* populations, analyzing the polytene chromosome in the larval salivary glands (Martin, 1962; Atchley & Davis, 1979). Overall, six heterozygous inversions were found: one on chromosome I, two on chro-

somosome II, and three on chromosome III. Three inversions were frequently found in the 1182 specimens analyzed, which were obtained from 18 sites—inversion A (chromosome II) was found in 51.8% of the individuals, inversion B (chromosome II) was in 37.8%, and inversion C (chromosome III) was in 13.2%. Clinality was observed for inversion B, and the frequency of inversion C varied among populations (Atchley & Davis, 1979). No statistically significant relationships were detected between ecological conditions (pH, electrical conductivity of the medium, relative organic content, and species composition of the vegetation) and the heterozygosity levels of chromosome variants (Atchley & Davis, 1979). Michailova *et al.* (2021) show the stability three from five inversions described Atchley and Davis (1979) in the population *B. antarctica* Wiencke Island, 500 m to SW from Port Lockroy.

Events such as dispersal to new habitats/areas or altered environmental conditions are followed in different Diptera species by changes in the frequencies and types of chromosomal rearrangements (Garcia & Valente, 2018). Unfortunately, the polytene chromosomes of *B. antarctica* have not been studied since the original publications, and the current state of the chromosomal polymorphism in different populations is unknown. Likewise, there are no reports of the species' karyotype based on its nonpolytene chromosomes, except for that of Martin (1962), who mentioned that chromosome number verification ($2n = 6$) was based on the somatic metaphase.

Belgica antarctica genome

Bioinformatic studies of genomes are widely used to clarify the genetics underlying adaptation mechanisms. Currently, the genomes of two Antarctic Chironomidae have been sequenced—those of *B. antarctica* and *Parochlus steinenii* (Kelley *et al.*, 2014; Kim *et al.*, 2017). At the time of sequencing, the genome of *B. antarctica* appeared to be strikingly small at only 99.25 Mbp for females and 98.4 Mbp for males, which are amongst the smallest of all analyzed insect genomes (Kelley *et al.*, 2014). However, as studies of other species' genomes have become available, it is now clear that Chironomidae genomes are generally not large (Table 1), and species of the subfamily Orthocladiinae to which *B. antarctica* belongs generally have the smallest genomes in the family (Cornette *et al.*, 2015). The genome of *P. steinenii* has a total size of 138 Mbp (Kim *et al.*, 2017). The genomes of *E. murphyi* and *B. albipes* have not yet been sequenced or analyzed.

Table 1 Features of the assembled genomes of some Diptera.

Species	Genome size (Mbp)	GC content	Number of genes	Repeat sequences (%)	Intron length (bp)	Reference
<i>Belgica antarctica</i>	98–99	0.39	13 517	0.12	309	Kelley <i>et al.</i> , 2014
<i>Parochlus steinenii</i>	138	0.322	13 468	0.48	319	Kim <i>et al.</i> , 2017
<i>Chironomus tentans</i>	~200	0.312	15 120	10	1103	Kutsenko <i>et al.</i> , 2014
<i>Chironomus riparius</i>	~200	0.311	13 449	9.14	ND	Schmidt-Ott <i>et al.</i> , 2009; Schmidt <i>et al.</i> , 2020
<i>Polypedium vanderplanki</i>	104	0.28	17 137	5.01	533	Gusev <i>et al.</i> , 2014
<i>Polypedium nubifer</i>	107	0.39	16 553	3.30	452	Gusev <i>et al.</i> , 2014
<i>Clunio marinus</i>	86	0.317	14 041	ND	ND	Kaiser <i>et al.</i> , 2016
<i>Aedes aegypti</i>	1 376	0.382	15 419	47	4685	Nene <i>et al.</i> , 2007
<i>Drosophila melanogaster</i> (Genome Release 4)	180	0.425	~13 600	20	1175	Adams <i>et al.</i> , 2000

ND: no data.

Such a small genome size was initially unexpected for *B. antarctica* because the requirements of life at low temperatures were intuitively associated with larger genome sizes (Hessen *et al.*, 2013; Kelley *et al.*, 2014). However, Kelley *et al.* (2014) offered an alternative hypothesis, proposing that a small genome could be a specific adaptation to severe habitat conditions. However, although the available data show that the genomes of Chironomidae are typically small, that of *B. antarctica* is still particularly small and whether this is dictated by evolutionary descent or facilitates stress tolerance remains inconclusive (Cornette *et al.*, 2015).

The number of functional genes in *B. antarctica* is generally similar to that of other Diptera. Approximately 19.4% of the genome consists of protein-coding genes, of which 13 517 are known. Interestingly, *P. steinenii* has a considerably larger genome but essentially the same number of genes at 13 468 (Kim *et al.*, 2017). The smaller genome size of *B. antarctica* is achieved through a decrease in the number of repeats, fewer transposable elements (transposons and retrotransposons; TEs), and shortened introns (Kelley *et al.*, 2014). *Parochlus steinenii* has approximately four times as many repeats as *B. antarctica* (Kim *et al.*, 2017). The *B. antarctica* genome only contains about 0.12% TEs, which is exceptionally few compared with other Chironomidae—for example, 0.49% for *P. steinenii* (Kim *et al.*, 2017), 10% for *Chironomus tentans* (Kutsenko *et al.*, 2014), and 47% for *Aedes aegypti* (Nene *et al.*, 2007) (Table 1). Even though *P. steinenii* has few TEs compared with other chironomids, the disparity in TE content does not explain the overall difference in the genome sizes of the two Antarctic species (Kim *et al.*, 2017). The TEs of different classes are reduced unevenly, potentially indicating that only selected TE superfamilies are important in the *B. antarctica* genome. *Belgica antarctica* has 154 TE families representing all main superfamilies.

One reason proposed for low TE content is the constitutively high expression of *hsp90* in larvae, through its direct interaction with piRNA biogenesis or suppressive action on the activation of transposition (Specchia *et al.*, 2010; Kelley *et al.*, 2014). The low numbers of DNA transposons could also be connected to the generally assumed low frequencies of horizontal transfer in the Antarctic, owing to low species diversity present (Kelley *et al.*, 2014). In *Drosophila*, some TEs are associated with genes that have adaptive effects (Franchini *et al.*, 2004). Therefore, the presence of such adaptive insertions might still be predicted in *B. antarctica* even given the low number of TEs in its genome.

In both *B. antarctica* and *P. steinenii*, intron length variability is low (309 and 319, respectively) (Kim *et al.*,

2017). However, in *P. steinenii*, the number of introns per gene is significantly greater than in *B. antarctica* and other Diptera (Kim *et al.*, 2017). Small introns are generally typical of chironomids, but just as with TEs, they are much shorter in the two native Antarctic insects, which provides some support to the hypothesis of selection for overall genome size reduction in insects of extreme Antarctic environments (Kelley *et al.*, 2014).

Overall, the genome of *B. antarctica* is typical of the Chironomidae, despite its small size, the presence of very few TEs, and the short intron lengths compared with those of the family in general. The species' adaptation to Antarctic conditions cannot, therefore, be explained by its general genome characteristics and, rather, the particulars of specific genes and their regulatory mechanisms must be examined.

Understanding mechanisms of adaptation demands the elucidation of their molecular basis. Genomic analyses therefore create new opportunities for studying these mechanisms, including the search for adaptive loci and gene duplications. High-throughput sequencing technologies now allow both affordable and time-efficient examination of whole-genome patterns of genetic diversity within and between populations and identification of those genes that show signatures of recent positive selection and/or divergent adaptation. Analyses of genomes representing different populations will not only allow the identification of genes responsible for local adaptation(s) in specific regions (e.g., in Antarctic insects), but also discovery of adaptive phenotypes and the selective pressures acting on them (Radwan & Babik, 2012).

Evolutionary and phylogenetic studies

The evolutionary placement of *B. antarctica* within Chironomidae was originally assessed based on *28S rRNA* gene sequences. Today, using this marker alone is deemed insufficient, and nucleotide sequences of ribosomal genes and internal transcribed spacers from the ITS1–5.8S rRNA–ITS2a–2S rRNA–ITS2 region of the rRNA gene cluster are proposed to be required for species identification and reconstruction of the phylogeny of the family Chironomidae (Gunderina & Katokhin, 2020). Among the species subjected to molecular study to date, the most closely related to *B. antarctica* is *E. murphyi* (Allegrucci *et al.*, 2006). Indeed, Allegrucci *et al.* (2012) concluded that *B. antarctica* is more closely related to *E. murphyi* than to *B. albipes*, an observation that led those authors to suggest that *E. murphyi* be considered a member of the genus *Belgica*. However, further studies testing more markers are required to confirm the

phylogenetic relationships between these three species. The best estimate of the divergence of *B. antarctica* and *E. murphyi* is approximately 49 Ma, following separation from the closely related genus *Clunio* (currently present in South America) 68.5 Ma. Cranston (1985) placed *B. antarctica* in the “*Clunio* group” of the subfamily Orthocladiinae. These data are broadly consistent with current reconstructions of the final stages of the breakup of Gondwana with the separation of southern South America and the Antarctic Peninsula, as well as the isolation of the tectonic microplate containing South Georgia (to which *E. murphyi* is endemic) (Convey *et al.*, 2018).

Using *coxI* mtDNA sequences, Allegrucci *et al.* (2012) found that 12 populations of *B. antarctica* clustered into 16 haplotypes (based on 48 differing sites) and were themselves clearly grouped into four haplogroups (A–D). Three of the four haplogroups included various populations from geographically close locations, while haplogroup C was found uniquely on Goudier Island, surrounded by islands inhabited by haplogroup D.

As noted above, interpopulation differences in *B. antarctica* were first demonstrated by Atchley and Davis (1979). Their study was based on material from 18 sites on islands all in the vicinity of Anvers Island and thus sampled only a small portion of the species' range. More significant chromosome aberration-level differences might therefore be expected across the entire distribution range, which extends across roughly six degrees of latitude (approximately 63–69°S; c. 720 km) along the South Shetland Islands and western coastal regions of the Antarctic Peninsula. Given that large inversions are difficult to detect by analyzing full genome sequences, a study of inversion polymorphism with the use of polytene chromosomes would still be of value.

The relationships between the two native Antarctic terrestrial insect species and their various sub-Antarctic and South American relatives are yet to be fully clarified and require further research using various genetic markers and novel methodologies.

Stress resistance

For Antarctic arthropods, stress resistance is an important prerequisite for survival. Stress resistance is thus one of the most widely studied aspects of the biology of Antarctic arthropods and other invertebrates (Cannon & Block, 1988; Convey, 1996; Peck *et al.*, 2006; Denlinger & Lee, 2010; Convey *et al.*, 2014; Teets & Denlinger, 2014; Everatt *et al.*, 2015). The primary environmental stress challenges facing *B. antarctica* and its close relative *E. murphyi* are low temperatures, desiccation, and

osmotic stress from exposure to seawater (Baust & Edwards, 1979; Baust & Lee, 1983, 1987; Bartlett *et al.*, 2020, 2021).

Cold and freezing stress

Low temperatures are a powerful stressor limiting the survival and distribution of terrestrial invertebrates in Antarctica. Larvae of the Antarctic midge are freeze tolerant year-round (Kawarasaki *et al.*, 2014a), but the success of the strategy depends on other features of their local microhabitats, because they are susceptible to inoculative freezing damage in wet environments (Elnitsky *et al.*, 2008). Although their limited supercooling capacity (between -6°C and -8°C) is sufficient to survive the year-round temperatures in many maritime Antarctic habitats buffered by winter snow cover, it is still relatively limited (Convey *et al.*, 2018). Rather, the larvae can tolerate controlled ice formation in extracellular fluids (Baust & Lee, 1987) and survive temperatures as low as -20°C in this state (Teets *et al.*, 2008; Teets & Denlinger, 2014). While winter-acclimated larvae survive freezing and supercooling equally well, freezing results in slightly greater energetic costs and elevated expression of certain heat shock proteins (Teets *et al.*, 2020). Freezing tolerance varies seasonally, with larvae collected during summer dying at higher temperatures on average (-6°C) than those collected in winter (-12°C) (Lee *et al.*, 2006). Their lower thermal limit remains unchanged at approximately -13°C throughout the year (Baust & Lee, 1987), although a more recent study has reported larval survival to approximately -20°C (Lee *et al.*, 2006; Teets *et al.*, 2008). The larvae are also capable of rapid cold hardening (Kawarasaki *et al.*, 2019), a form of phenotypic plasticity that allows ectotherms to quickly enhance cold tolerance in response to a brief initial chilling stimulus (lasting minutes to hours; Worland & Convey, 2001). This feature has not been widely studied or observed in freeze-tolerant insects (Lee *et al.*, 2006; Teets *et al.*, 2008). At the biochemical level, when larvae were frozen (at -10°C for 6 hours), concentrations of alanine and aspartate increased along with urea. Freezing also led to increased concentrations of three polyols (glycerol, mannitol, and erythritol) and a reduction in serine, potentially identifying this amino acid as a marker for stress in this species (Michaud *et al.*, 2008).

Desiccation stress

Water availability in the liquid state is one of the greatest environmental challenges for insects and other in-

vertebrates in Antarctica (Kennedy, 1993; Convey *et al.*, 2014). Water is frozen for much of the year. Therefore, invertebrates tolerate desiccation stress either by lowering their body water content, surviving in a dehydrated state, or by harnessing their freezing resistance mechanisms (Teets & Denlinger, 2014; Everatt *et al.*, 2015; Chown & Convey, 2016). Larvae of *B. antarctica* can tolerate rapid water loss and high levels of dehydration (Teets & Denlinger, 2014), surviving even with up to 70% water loss (Benoit *et al.*, 2007; Hayward *et al.*, 2007). They are also able to withstand at least four dehydration–rehydration cycles following natural humidity fluctuations (Teets *et al.*, 2012a). Adult flies are relatively less tolerant to dehydration than the larvae (32% water loss for females and 34% for males) (Benoit *et al.*, 2007).

A particular strategy of combined cold and desiccation tolerance, which is exhibited by some Antarctic and Arctic invertebrates, is cryoprotective dehydration (Holmstrup & Sømme, 1998; Worland *et al.*, 1998; Holmstrup, 2002). The adaptive importance of this strategy for small arthropods lies in rapid water loss in response to subzero temperatures in the presence of ice (Sorensen & Holmstrup, 2011), facilitated by a permeable cuticle, very strong resistance to dehydration, and the ability to resist inoculative freezing (Teets & Denlinger, 2014; Kawarasaki *et al.*, 2014b). Cryoprotective dehydration in insects was first confirmed for the larvae of *B. antarctica*, and they can utilize both freezing tolerance and cryoprotective dehydration strategies (Elnitsky *et al.*, 2008; Benoit *et al.*, 2009; Teets *et al.*, 2011, 2012a; Kawarasaki *et al.*, 2014a).

The processes of dehydration and rehydration occur in *B. antarctica* larvae with the help of proteins known as aquaporins (AQPs), a family of cell membrane proteins of approximately 30 kDa molecular mass. They are characterized by homology at the amino acid sequence level and thus may have similar three-dimensional structures. AQP proteins are situated in the cell membrane and form water channels. Some AQPs also allow the passage of glycerol, urea, and other small nonpolar molecules (Jung *et al.*, 2020). *Belgica antarctica* possesses AQPs belonging to five different subfamilies (Goto *et al.*, 2011, 2015), including DRIP-like protein (a *Drosophila* aquaporin), *Pyrocoelia rufa* integral proteins (PRIP superfamily), Big Brain proteins (BIB), *Rhodnius prolixus* integral proteins (RPIP), and *Lygus hesperus* integral protein (LHIP). Yi *et al.* (2011) reported AQP-like proteins in different tissues of *B. antarctica* larvae. Inhibition of AQPs in *B. antarctica* disrupts the normal dehydration processes (Yi *et al.*, 2011).

Freezing avoidance and freezing tolerance strategies involve the accumulation of cryoprotectants. Freeze

tolerance (the control of ice formation within the organism) is facilitated by antifreeze proteins (AFPs), protecting the organism from damage at the cellular and tissue levels. AFPs were identified in Lake Ontario midges (Basu *et al.*, 2015). The five arthropod AFP subfamilies are not homologous (Cheung *et al.*, 2017). This diversity in AFP sequence and structure suggests that AFPs arose recently and independently from different progenitors, developing the same function through convergent evolution. Therefore, *B. antarctica*, through its geographical distribution and its taxonomic position, potentially possesses these proteins (Convey & Block, 1996; Basu *et al.*, 2015).

Osmotic and other environmental stresses

The responses of *B. antarctica* to osmotic stress from seawater and other types of stress have been studied much less than have the species' tolerance to cold and dehydration. In summer, the insect's shoreline habitats can be exposed to sea spray during storms (Baust & Lee, 1987). Snowmelt and precipitation, increasingly as rain, may dilute this marine input, although dry conditions and water evaporation can also lead to hyperosmotic stress. Further marine influence is possible in habitats where the species occurs that are strongly influenced by penguin and seal concentrations on land, which introduce both seawater and exposure to high nutrient concentrations from their guano (Bokhorst *et al.*, 2019). Thus, tolerance of varying salt concentrations is critical for *B. antarctica* (Elnitsky *et al.*, 2009). The fly's larvae can survive in freshwater for up to 28 days and in undiluted seawater for up to 10 days (Baust & Lee, 1987; Elnitsky *et al.*, 2009). They exhibit tolerance to saltwater with 29.2% NaCl. High lethality is seen only after a week's exposure to 58.4% NaCl (Baust & Lee, 1987). Similar tolerance to saline and, to a lesser extent, hypersaline conditions is seen in the final-instar larvae of *E. murphyi* (Bartlett *et al.*, 2021). Exposure to seawater leads to decreased body water content and increased content of the osmolytes glucose and trehalose (Elnitsky *et al.*, 2009). Furthermore, brief exposure to various environmental stressors (e.g., hyperosmotic challenge, hypoosmotic challenge, pH change, UV irradiation) increase tolerance to further stresses in the larvae (Gantz *et al.*, 2020), an illustration of the "cross tolerance" phenomenon (Everatt *et al.*, 2015).

Another form of stress *B. antarctica* larvae commonly experiences is pH fluctuation (Baust & Lee, 1983; Gantz *et al.*, 2020). In their natural microhabitats, larvae have been found in conditions with pH ranging from 4 to 7.8 (Baust & Lee, 1983). Baust and Lee (1987) demonstrated

that the larvae could survive for more than 2 weeks at a pH range of 3–12, and 90% of larvae could survive 4 weeks at pH 6–11.

Antarctic terrestrial organisms may also experience high levels of ultraviolet irradiation and, as a result, require high tolerance to oxidative stress (Lopez-Martinez *et al.*, 2008). *Belgica antarctica* larvae show relatively high antioxidant capacity—five times greater than that of *Eurosta solidaginis*, a gall fly found in temperate latitudes that is also freeze tolerant. The antioxidant capacity of adults, active on the soil and vegetation surface at the warmest period of summer, is also more than 1.5 times greater than that of the larvae. Antioxidant capacity is unaffected by freezing, anoxia, or heat stress. Expression of superoxide dismutase (SOD) is high in larvae but absent in adult insects (Lopez-Martinez *et al.*, 2008).

Gene expression in the extreme environment of Antarctica

Among the Antarctic and sub-Antarctic insects, *B. antarctica* has been studied the most at the molecular level, especially in terms of gene expression changes in response to physiological stress (Teets & Denlinger, 2014).

Changes in expression of heat shock proteins

Heat shock proteins (hsps; *hsp70*, *hsp90*, *smhsp*) are highly expressed throughout the entire larval stage of *B. antarctica*. This differentiates the species from many well-studied insects of tropical and temperate latitudes that typically have elevated synthesis of hsps only in response to stressors, and in which high hsp protein levels compromise the expression of other proteins in normal conditions (Feder *et al.*, 1992; Krebs & Feder, 1997; Rinehart *et al.*, 2006). The high expression levels of hsps in *B. antarctica* larvae during active development and growth is thus difficult to explain, requiring a mechanism that supports high expression and functional activity of hsps without disrupting normal metabolism and the production of other proteins. The larvae also do not increase hsp expression or become more thermotolerant under experimental heating or cooling, suggesting that their hsp protective system is constitutively active (Rinehart *et al.*, 2006). Unlike the larvae, adult *B. antarctica* exhibit a typical heat-shock reaction that is thermally activated (Rinehart *et al.*, 2006). Alternative hypotheses for the high expression of *hsp* genes in *B. antarctica* larvae include protection from stressors such as low substrate pH, low temperature, and anoxia (Baust & Lee, 1987;

Kabakov & Gabai, 1993; Vidair *et al.*, 1996; Chen *et al.*, 2002; Rinehart *et al.*, 2006).

The high-sustained *hsp* expression in the larvae of *B. antarctica* has been hypothesized to indicate a general adaptive feature for Antarctic species (Rinehart *et al.*, 2006). Similarly, *hsp70* is constitutively expressed in the Antarctic fish *Trematomus bernacchii* (Buckley *et al.*, 2004), the Antarctic ciliate *Euplotes focardii* (La Terza *et al.*, 2001), and other polar marine species (Place *et al.*, 2004; Clark *et al.*, 2008). *Candida psychrophila* (a yeast found in penguin guano) constitutively expresses *hsp70* and *hsp90* (Deegenars & Watson, 1997; Rinehart *et al.*, 2006). The high level of hsps in the larvae of *B. antarctica* and in the various marine species, all of which inhabit cold and thermally more stable habitats, suggest that hsps are used as chaperones that facilitate the folding process of other proteins in cold conditions and alleviate the effects of oxidative stress and dehydration (Rinehart *et al.*, 2006; see also reviews of Clark & Worland, 2008; Clark & Peck, 2009). The genes encoding hsps are strongly expressed over a long period during diapause in temperate insects (Denlinger *et al.*, 2001; Rinehart *et al.*, 2006). However, in this circumstance, *hsp* gene expression halts the development of the body (Denlinger *et al.*, 2001; Rinehart *et al.*, 2006). The gene expression patterns of hsps also differ between *B. antarctica* and temperate species, with high levels of *hsp90* expression, which, in temperate insects, is either inhibited during diapause (Rinehart & Denlinger, 2000; Rinehart *et al.*, 2006) or not produced (Tachibana *et al.*, 2005; Yocum *et al.*, 2005; Rinehart *et al.*, 2006).

Gene expression changes during cryoprotective dehydration

The process of cryoprotective dehydration has a considerable impact on gene expression in *B. antarctica*—of 11 500 studied genes, the expression of 3275 changed under dehydration, and 2365 were affected by cryoprotective dehydration. Although some of the genes differed between the two processes, the considerable majority (1909) were shared (Teets *et al.*, 2012b). Among the genes whose expression was upregulated during dehydration were the *hsp* families *smhsp* (three proteins), *hsp40* (two proteins), *hsp70* (eight proteins), and *hsp90* (one protein) (Teets *et al.*, 2012b). The transcription factor *hsf*, which regulates *hsp* expression, has also been reported to be upregulated (Morimoto, 1998; Teets *et al.*, 2012b).

Hsps serving as chaperones direct damaged proteins into the proteasome, which prevents the accumulation of nonfunctional proteins, cyclopeptides, and amino acids in

the cell (Goldberg, 2003). Teets *et al.* (2012b) also reported that genes linked to ubiquitin-dependent proteolysis were upregulated. This suggests coordinated regulation of the *hsp* genes and proteasome genes, both of which function to restore and degrade damaged proteins during dehydration.

Desiccation may also activate the autophagy pathway (Teets *et al.*, 2012b). Autophagy is a catabolic process in which parts of the cytoplasm and organelles are digested in lysosomes (Alberts *et al.*, 2007), which thus conserves cell macromolecules and energy during periods of stress and nutrient deficiency (Maiuri *et al.*, 2007; Teets *et al.*, 2012b). Therefore, autophagy can provide an alternative pathway to programmed cell death as a result of stress, reducing the number of cells dying by converting cell components and inhibiting apoptotic cell death (Maiuri *et al.*, 2007; Teets *et al.*, 2012b). The latter study proposed that autophagy levels increase under dehydration, conserving energy and facilitating survival during prolonged cell stress, and reported the expression of 92 homologues of genes with known functions in autophagy and apoptosis during dehydration and/or cryoprotective dehydration.

Consistent with the need to conserve energy expenditure during dehydration, genes linked to general metabolism and ATP synthesis become downregulated. The larvae of *B. antarctica* exhibit significantly reduced oxygen consumption in response to dehydration (Benoit *et al.*, 2007; Teets *et al.*, 2012a). Metabolic depression is a common adaptation in desiccation-tolerant insects, probably minimizing loss of water through respiration and of water bound to glycogen and other carbohydrates (Marion *et al.*, 2003; Teets *et al.*, 2012a).

The available gene expression and metabolomics data are generally consistent. However, some changes in the metabolome are not directly related to changes in transcription, but rather by posttranscriptional control. In some cases, changes in gene expression can influence the rates of metabolic processes beyond those described by Teets *et al.* (2012b).

Lopez-Martinez *et al.* (2008) concluded that the expression of multiple genes was influenced by the hydration state of *B. antarctica* larvae. These primarily include genes encoding hsps (*smhsp*, *hsp70*, *hsp90*), antioxidant (superoxide dismutase, catalase) and detoxicant systems (metallothionein, cytochrome p450), cell membrane related machinery (fatty acid desaturase, protein activating phospholipase A2, acyl-Coa desaturase), and the cytoskeleton (actin, muscle specific actin). Other components such as V-ATPase, proteins with structural zinc finger motifs, and pacifastin (a serine peptidase inhibitor) are also affected by hydration state.

Conclusions

Belgica antarctica is an endemic insect living in the Antarctic whose range may be altered as its supporting habitats adjust to the region's shifting climate, as recently predicted in a modeling study of the second native Antarctic insect *Parochlus steinenii* (Contador *et al.*, 2020). Such events may drive changes in its mechanisms of adaptation to extreme conditions at multiple functional biological levels, which makes the species an excellent research model.

There are numerous studies available on the species' genomic, biochemical, physiological, and life-history survival mechanisms in the Antarctic. The species can be cultured in the laboratory, and its ecology, physiology, polytene chromosomes, sequenced genome, and both transcriptome and proteome are the focus of a robust and large body of literature. Considering the requirements for model organisms and the well-known examples among Diptera (*Drosophila melanogaster*, *Anopheles gambiae*), we propose that *B. antarctica* can, therefore, be viewed as a model organism for studying adaptation mechanisms to the challenges of extreme environments.

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Disclosure

The authors declare no conflict of interest.

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