

1 MIxS-SA: a MIxS extension defining the minimum information standard for sequence data
2 from symbiont-associated micro-organisms.

3 Running title: MIxS - symbiont-associated environmental package

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The symbiont-associated (SA) environmental package is a new extension to the minimum information about any (x) sequence (MIxS) standards, established by the Parasite Microbiome Project (PMP) consortium, in collaboration with the Genomics Standard Consortium. The SA was built upon the host-associated MIxS standard, but reflects the nestedness of symbiont-associated microbiota within and across host-symbiont-microbe interactions. This package is designed to facilitate the collection and reporting of a broad range of metadata information that apply to symbionts such as life history traits, association with one or multiple host organisms, or the nature of host-symbiont interactions along the mutualism-parasitism continuum. To better reflect the inherent nestedness of all biological systems, we present a novel feature that allows users to co-localize samples, to nest a package within another package, and to identify replicates. Adoption of the MIxS-SA and of the new terms will facilitate reports of complex sampling design from a myriad of environments.

Main text

Interspecific interactions are ubiquitous across the Tree of Life. With the realization that eukaryotic organisms can harbor rich microbial communities, came also the view that these smaller partners may in fact play important roles in mediating host-symbiont associations, thus adding a further layer to this complex set of nested interactions, *i.e.* host-symbiont-microbe [1-8]. As the number of studies exploring the microorganisms associated with symbiotic organisms increases, likewise does the need for compliant standardized metadata that provides contextual information associated with each study and sample. Standardized metadata allows for the integration of data across organisms, resources, and within data repositories. Here, we present

the symbiont-associated (SA) environmental package as a new extension to the minimum information about any (x) sequence (MIxS) standards [9], which will be included in MIxS version 6. Whilst the MIxS-SA expands upon the MIxS host-associated environmental package [9], it reflects the need for a new standard that takes into account the distinct life history traits of symbionts, their association with one or multiple host organisms, the complex nature of host-symbiont interactions along the mutualism-parasitism continuum, and the nestedness of symbiont-associated microbiota. We also propose adding the term ‘relationship to other packages’ to all environmental packages across the domains of life, to allow for integrated analysis of symbiont and host microbiota by linking metadata elements across environmental packages. This will allow users to nest a package within another package, and to identify replicates. This added feature is pivotal for the study of the microbiome of symbionts that are themselves nested within a host, reflects the inherent nestedness of all ecosystems and will facilitate reports of complex sampling design from a myriad of environments.

Collecting relevant metadata (data describing data) is now widely recognized as critical to contextualize samples and increase their reusability and reproducibility [9-11]. The Genomic Standards Consortium (GSC, <https://gensc.org>) has developed and maintains a suite of minimal information metadata standards for describing sequence metadata (checklists) for genome (MIGS), metagenome (MIMS), marker gene sequences (MIMARKS), simple amplified genome (MISAG), metagenome-assembled genome (MIMAG), virus genomes (MIUViG) and environmental packages for describing habitat-specific contextual data of the sampling environment [9,10,12,13], collectively referred to as the Minimum Information about any (x) Sequence (MIxS) standard (ref. 9, <https://gensc.org/mixs/>).

107 The MIxS standards are used broadly across the microbiome research communities. These
108 standards have been integrated into large scale microbiome projects (e.g. Human Microbiome
109 Project, <https://www.hmpdacc.org/>), Earth Microbiome Project (<https://earthmicrobiome.org/>),
110 Microbiology of the Built Environment (MoBE, <https://www.microbe.net>), microbiome
111 bioinformatics platforms (e.g. QIIME, Qiita, mothur, JGI GOLD, MG-RAST, EBI, NCBI) and
112 are now required upon manuscript submission. A primary advantage of the MIxS standards is the
113 collation of large aggregates of associated metadata that can be harnessed to uncover, and
114 eventually comprehend, patterns of microbial diversity and ecology.

115

116 The MIxS-SA package was initially drafted during the 1st Parasite Microbiome project workshop
117 that involved the contribution of members of the GSC in addition to microbial ecologists,
118 parasitologists, pathologists and marine biologists [14]. Participants rapidly identified the need to
119 incorporate information on the nestedness of symbiont-associated systems, and the absence
120 within the MIxS host-associated package of descriptors of complex life histories of mutualistic
121 and parasitic symbionts. Until now, researchers have either omitted this information or added
122 research-specific symbiont-associated annotations, limiting significantly the potential to
123 compare, combine and/or reuse data from different systems and studies. Whereas the MIxS-SA
124 package was initially designed for the study of parasite-microbes interaction, the scope of the
125 package was expanded to include non-parasitic symbionts. This addition is a necessary
126 expansion due to the context-dependent nature of symbiotic interactions and the ability of a
127 given symbiont to interact differently with different organisms. Notably, the resulting MIxS-SA
128 package reduces the need to develop additional highly similar packages for different types of
129 symbionts.

130

131 Symbiotic associations are generally classified as mutualistic (mutually beneficial association),
132 commensal (beneficial association to one of the partners, but not harmful to the other), or
133 parasitic (detrimental association to one of the partners) [15]. In the context of the symbiont-
134 associated package, the term symbiont applies to macro and microorganisms that can establish a
135 physical interaction with at least one other organism at some stage of their life cycle regardless
136 of the nature and dependence of the interaction. As such, this definition also covers symbiotic
137 organisms that establish facultative and accidental associations (e.g. dead-end hosts), not
138 requiring evolutionary processes to explain their association, but excludes free-living organisms
139 that establish a symbiotic relationship with another free-living organism (e.g. flowers and bees).
140 The MxS-SA package presented herein has gone through an open and iterative review process
141 engaging the GSC community and experts studying symbiotic organisms across various
142 symbiont and host taxa.

143 Here, we present the selected list of metadata descriptors for symbiont-associated microbiota
144 studies, including a subset of mandatory (M) terms that underpin metadata compliance (Table 1;
145 Supplementary Information SI-1 contains all MxS-SA items). In order to allow comparative
146 studies of the microbiota of, sometimes closely related, free-living and symbiotic organisms, the
147 MxS-SA includes terms already found in the MxS host-associated package. Thus, in MxS-SA,
148 the term "host" (when used alone) refers to the host of the biological sample which is the
149 symbiotic organism. New terms were created to characterise the "host of the symbiotic host".
150 We provide symbiont-associated package specific "Expected values" and "Examples". Changes
151 to the package (addition of terms, modification etc.) can be proposed by the community by

152 creating a ticket on the MIxS GitHub page
153 (<https://github.com/GenomicsStandardsConsortium/mixs>).
154
155 Given the diversity of symbiotic interactions and that the nature and dependence of such
156 interactions can be context-dependent rather than a fixed trait, it was crucial to define terms and
157 provide value syntax that were inclusive for diverse types of symbioses and also across the
158 symbiont life histories and transmission processes. For example, the term “host dependence” (a
159 mandatory item) and “type of symbiosis” (a conditional item) are discrete but complementary
160 items. While “host dependence” aims to provide a general characterization of the known type of
161 host dependence for the symbiotic organism (e.g. facultative), “type of symbiosis” was
162 specifically designed to further characterize the type of biological interaction established
163 between the symbiotic organism and its respective host at the moment the biological sample was
164 taken (e.g. mutualistic). As a result, the MIxS-SA package features mandatory and conditionally
165 mandatory, and optional features that enable flexibility according to the knowledge of the study
166 system at the time of sampling. Two examples of MIxS-SA-compliant metadata are provided in
167 Supplementary information (SI-2), and the respective study designs are presented in Figure 1.
168 The examples refer to 16S rRNA gene studies of (a) the bacterial communities of the parasite
169 *Coitocaecum parvum*, a trematode, across four of its life stages: the sporocyst, the metacercaria
170 and the adult, as well as the free-living cercaria [16], and (b) of the leaves and roots of the
171 parasitic plant *Orobanche hederæ* and its ivy host, *Hedera* spp. [17].

172 While identical terms are often used in several of the 17 environmental packages currently
173 available (<https://gensc.org/mixs/>), here we introduce three additional new terms: one is shared
174 by several relevant MIxS environmental packages, and the two others will feature within the core

175 MlXS package. The new term “observed host symbionts” provides a more comprehensive
176 descriptor for the subject organism associations with smaller symbionts and it has been added to
177 the host-associated, human-associated, plant-associated, human-vaginal, human-skin, human-
178 oral and human-gut packages. The term “biotic relationship” has been added to the core package
179 as a conditional descriptor of the relationship between the subject organism and other larger host
180 organism(s). Finally, it appears necessary to include in the MlXS core a new term that takes into
181 account the nested feature of most associations found in nature, such as host-symbiont-
182 microorganism, in which multiple packages are necessary to describe the samples of the study
183 (e.g. water, sediment, host-associated, and symbiont-associated). The proposed term
184 “relationship to other samples” indicates the direct relationship between two samples from the
185 same Bioproject, that are described in different environmental package(s). This proposed feature,
186 still under development, will allow for integrated analyses of the microbiota of symbiotic
187 organisms and their direct environment, even in the context of co-infections (e.g. symbiont-
188 associated SA1234 is ‘within’ host-associated sample HA8974, ‘next to’ symbiont-associated
189 sample SA7890). This feature will also benefit other studies by providing ecologically-relevant
190 contextual information (e.g. host-associated HA2567 is ‘within’ environmental water sample
191 W1234, ‘next to’ host-associated sample HA5679, ‘next’ to environmental soil sample S5897).
192 In conclusion, it is our hope that the MlXS-SA, together with the new terms, will enable
193 researchers to better conduct integrated analyses of multi-level biological systems with the
194 ultimate goal of better understanding the role of microbes associated with symbionts.

195 **Conflict of Interest**

196 The authors declare no conflict of interest.

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209 **Author contributions**

210 NMD conceptualized the MxS package. All authors contributed to the writing and design of the
211 MxS-SA package. F.J. and N.M.D. prepared the manuscript. All authors agreed with the final
212 version of the package and manuscript.

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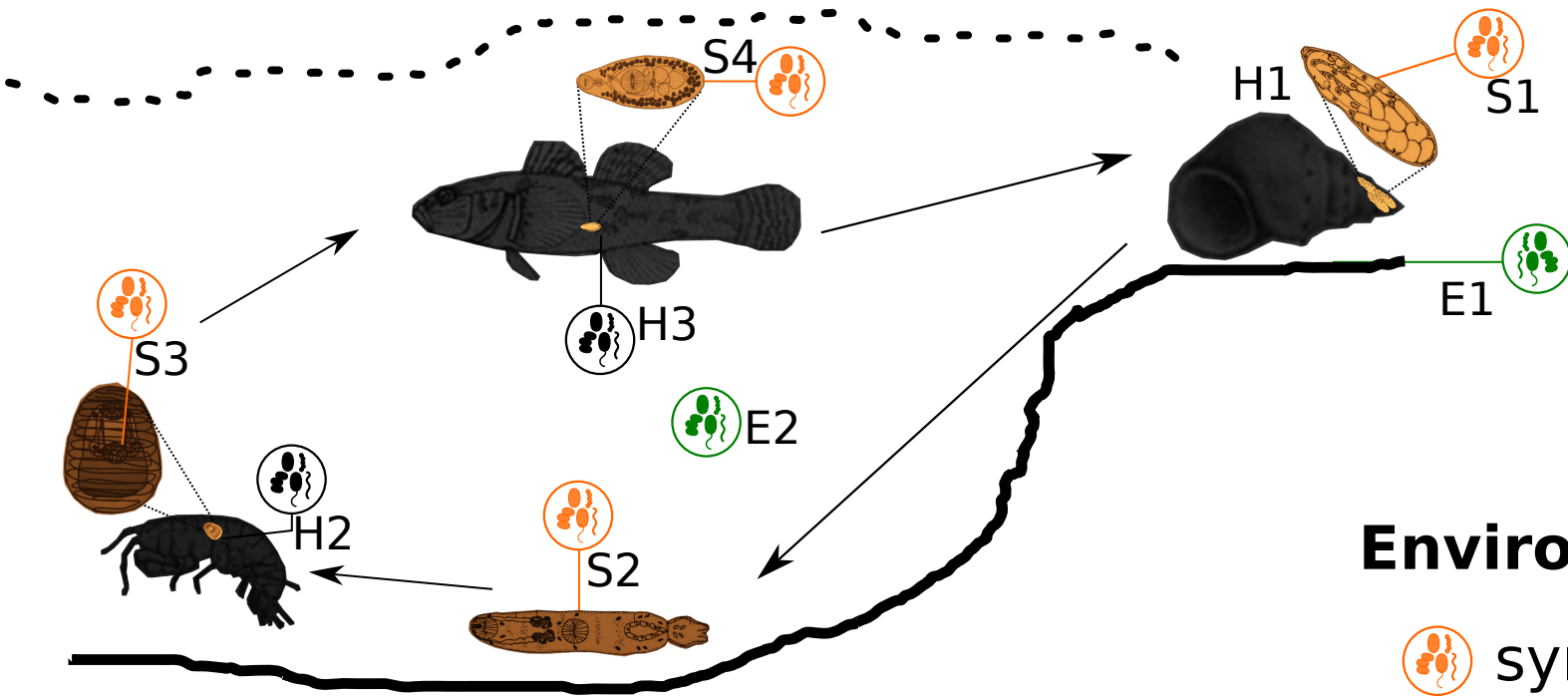
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257 **Figure legend:**

258 **Fig. 1 Examples of study design for the sampling of microbes of symbiotic organisms, their**
259 **hosts and environment. a,** Trematode *Coitocaecum parvum* different life stages (S1, S2, S3, S4)
260 are reported with the MlXS-SA package. The microbiome of infected snail (H1), amphipod (H2)
261 and fish (H3) hosts are reported with the MlXS host-associated package. In addition, the
262 microbiome of environmental sediment (E1) and water (E2) from which these organisms were
263 collected can be reported with MlXS-sediment and MlXS-water, respectively. The following
264 relationships are reported: S1 ‘within’ H1, S2 ‘within’ E2, S3 ‘within’ H2, S4 ‘within’ H3, H1
265 ‘next to’ E1, H1 ‘within’ E2, H2 ‘within’ E2, H3 ‘within’ E2, E1 ‘next to’ E2. **b,** Angiosperm
266 *Orobancha hederac* (S5, S6) parasitizing a host plant (P1, P2, P3) is reported using the included
267 MlXS-SA and MlXS-PA (plant-associated) packages respectively. In addition, the MlXS-soil
268 package is used to report corresponding soil samples. The following relationships are reported:
269 S6 ‘within’ P1, S5 ‘next to’ S6, P1 ‘next to’ P2, P1 ‘next to’ P3, P2 ‘next to’ P3, P1 ‘within’ E3,
270 P3 ‘within’ E4, E3 ‘next to’ E4.

a

Environmental packages



symbiont-associated
(S1, S2, S3, S4, S5, S6)



host-associated
(H1, H2, H3, H4, H5)



plant-associated
(P1, P2, P3)



sediment (E1)



water (E2)



soil (E3, E4)

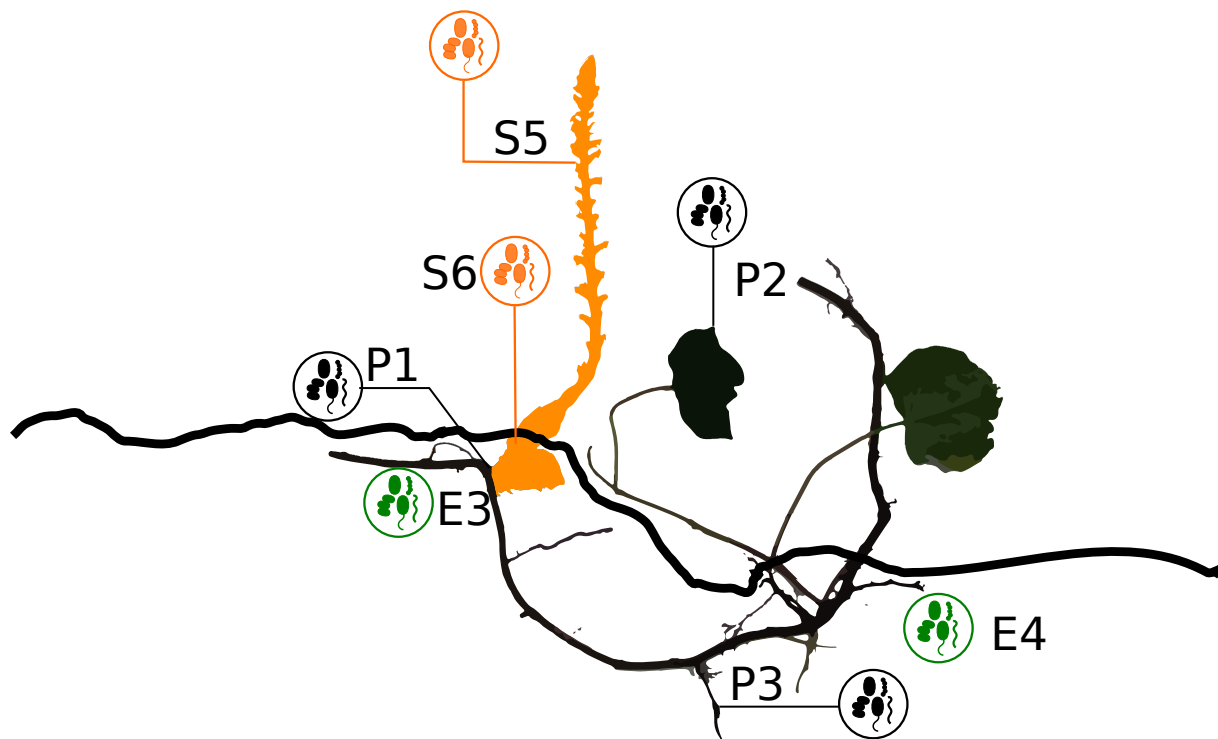
b

Table 1

Table 1 MxS symbiont-associated environmental package representative

MxS Package	Metadata category	Package item	Req
	Symbiont specific descriptors	host dependence	M
		type of symbiosis	
			C
		symbiotic host organism	
		life cycle type	
			M
		host life stage	M
		mode of transmission	
			C
		route of transmission	
	Symbiont – host relationship descriptors		O
		host number individual	
			O
		observed host symbionts	
			O
		host specificity	
			C
		host of the symbiont role	
			C
		host cellular location	
			C
		duration of association with the host	
			O

Symbiont-
associated

Table 1

**observed coinfecting
organisms in host of host**

		O
Host of the symbiont descriptors	host of the symbiotic host common name host of the symbiotic host local environmental context	O

		O
	host of the symbiotic host environmental medium	

		O
	host of the symbiotic host taxon id	O
	host of the symbiotic host subject id	O

Table 1

Relationship to other samples^a		
Core	Biotic relationship	C
<hr/>		
Observed host symbionts		C
<hr/>		
Other ^b		O

a, Term may be slightly amended when included to all packages.

b, Term added to host-associated, human-associated, plant-associated, human-
Req, Requirements: O, optional; C, Conditional; M, mandatory; TBD, to be de

Table 1

terms, along with requirement status, description and MIXS IDs.

Definition	MIXS ID
Type of host dependence for the symbiotic host organism to its host	0001315
Type of biological interaction established between the symbiotic host organism being sampled and its respective host	0001307
Type of life cycle of the symbiotic host species (the thing being sampled). Simple life cycles occur within a single host, complex ones within multiple different hosts over the course of their normal life cycle.	0001300
Description of life stage of host.	0000251
The process through which the symbiotic host organism entered the host from which it was sampled.	0001312
Description of path taken by the symbiotic host organism being sampled in order to establish a symbiotic relationship with the host (with which it was observed at the time of sampling) via a mode of transmission (specified in mode_transmission).	0001316
Number of symbiotic host individuals pooled at the time of collection.	0001305
The taxonomic name of the organism(s) found living in mutualistic, commensalistic, or parasitic symbiosis with the specific host. For cases when the specific host of the sample is a symbiont this field should refer to other organisms it is associated with. E.g.: hyperparasite species X (parasite of the parasite)	0001309
Level of specificity of symbiont-host interaction: e.g. generalist (symbiont able to establish associations with distantly related hosts) or species-specific.	0001308
Role of the host in the life cycle of the symbiotic organism.	0001303
The localization of the symbiotic host organism within the host from which it was sampled: e.g. intracellular if the symbiotic host organism is localized within the cells or extracellular if the symbiotic host organism is localized outside of cells.	0001313
Time spent in host of the symbiotic organism at the time of sampling; relevant scale depends on symbiotic organism and study.	0001299

Table 1

<p>The taxonomic name of any coinfecting organism observed in a symbiotic relationship with the host of the sampled host organism. e.g. where a sample collected from a host trematode species (A) which was collected from a host_of_host fish (B) that was also infected with a nematode (C), the value here would be (C) the nematode {species name} or {common name}. Multiple co-infecting species may be added in a comma-separated list. For listing symbiotic organisms associated with the host (A) use the term Observed host symbiont.</p>	0001310
<p>Common name of the host of the symbiotic host organism</p> <p>For a symbiotic host organism the local anatomical environment within its host may have causal influences. Report the anatomical entity(s) which are in the direct environment of the symbiotic host organism being sampled and which you believe have significant causal influences on your sample or specimen. For example, if the symbiotic host organism being sampled is an intestinal worm, its local environmental context will be the term for intestine from UBERON (http://uberon.github.io/).</p>	0001324
<p>Report the environmental material(s) immediately surrounding the symbiotic host organism at the time of sampling. This usually will be a tissue or substance type from the host, but may be another material if the symbiont is external to the host. We recommend using classes from the UBERON ontology, but subclasses of 'environmental material' (http://purl.obolibrary.org/obo/ENVO_00010483) may also be used. EnvO documentation about how to use the field:</p> <p>https://github.com/EnvironmentOntology/envo/wiki/Using-ENVO-with-MiXs . Terms from other OBO ontologies are permissible as long as they reference mass/volume nouns (e.g. air, water, blood) and not discrete, countable entities (e.g. intestines, heart).</p>	0001325
<p>NCBI taxon id of the host of the symbiotic host organism.</p>	0001326
<p>A unique identifier by which each host of the symbiotic host organism subject can be referred to, de-identified, e.g. #H14.</p>	0001306
	0001327

Table 1

indicates the direct relationship with another sample from the same Bioproject. Accepted terms are: 'technical replicate of', 'after', 'before', 'next to', 'within', and 'contains'. Can be repeated to reveal relationship to many samples.	TBD
Description of relationship(s) between the subject organism and other organism(s) it is associated with. E.g., parasite on species X; mutualist with species Y. The target organism is the subject of the relationship, and the other organism(s) is the object	0000028
The taxonomic name of the organism(s) found living in mutualistic, commensalistic, or parasitic symbiosis with the specific host.	0001309

vaginal, human-skin, human-oral, and human-gut packages.
 :terminated